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on

CLASPIN PROTEINS AND METHODS OF USE THEREOF

by

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CLASPIN PROTEINS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

[0001] This application claims the benefit of priority under 35 U.S.C. § 119(e) of United States Provisional Application 60/241,246, filed October 17, 2000, incorporated herein in its entirety.

ACKNOWLEDGEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with Government support under Grant Number GM-43974, awarded by the National Institutes of Health. The Government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention relates generally to cell cycle progression and more specifically to molecules involved in regulating DNA replication checkpoints.

BACKGROUND OF THE INVENTION

[0004] Growth and division of cells require a cell cycle, that is, a regular sequence of processes. Two processes of the cell cycle are the accurate replication of DNA and the segregation of chromosomes to the two daughter cells during mitosis. When mitosis is not taking place, the cell is in an interphase period. Interphase is subdivided into three phases; a synthetic phase known as S-phase, when DNA synthesis takes place, and G1 and G2, gap phases that separate S-phase from mitosis. G1 is the gap after mitosis before DNA synthesis starts, and G2 is the gap after DNA synthesis is complete before mitosis and cell division. The mechanisms that insure alteration between S-phase and G-phases are critical to achieving cells of correct size and chromosome number. Most cells divide after replication of their chromosomes, and most cells replicate their chromosomes after completing cell division. Cells rely on checkpoints that link cell division processes and chromosome replication processes.

[0005] Checkpoint mechanisms guarantee that eukaryotic cells maintain genomic integrity during cell division by monitoring damaged or incompletely replicated. Such mechanisms ensure

that cell cycle progression is stalled until aberrant DNA structures or replication intermediates can be eliminated. An ultimate target of these control mechanisms is the Cdc2-cyclin B complex, also known as maturation or M-phase promoting factor (MPF). During interphase, the Cdc2 subunit of MPF is down-regulated by inhibitory phosphorylations on its Thr-14 and Tyr-15 residues. A regulatory system containing two inhibitory kinases, Mytl and Weel, and a stimulatory phosphatase, Cdc25C, controls the activity of Cdc2 through reversible phosphorylation of these residues. Checkpoint mechanisms prevent the activating dephosphorylation of Cdc2 at the G2/M transition unless two accurate copies of the genome are available for transmission to daughter cells.

[0006] Genetic studies in yeast have identified proteins that are involved in sensing information damaged and/or incompletely replicated DNA and transmitting this information to effector molecules that interact directly with the cell cycle control machinery. In fission yeast, the sensor proteins are currently thought to include Rad1, Rad3, Rad9, Rad17, Rad26, Hus1, and Crb2/Rhp9. Effector proteins include the kinases Chk1 and Cdsl as well as 14-3-3 proteins such as Rad24. Similar pathways are found in budding yeast, and homologues of many of these proteins have been identified in higher eukaryotes, including humans, *Xenopus*, *Drosophila,* and mice. The most well established function of Chk1 in yeast and vertebrates is mediation of the binding of l4-3-3 proteins to Cdc25, which results in its cytoplasmic localization.

[0007] Phosphoinositide kinase relatives in fission yeast (Rad3), budding yeast (Mec1), and vertebrates (Atm and Atr) play an essential role in signaling the presence of damaged and/or unreplicated DNA to downstream regulators. For example, in fission yeast, Chk1 and Cds1 cannot function normally in the absence of Rad3. Similarly, Mec1 is a critical regulator of Rad53, a Cds1 homologue in budding yeast. In vertebrates, Atm is an upstream regulator of Chk2/Cds1. Atr is essential for genomic stability and early embryonic viability.

[0008] Despite these insights about certain components of checkpoint mechanisms, relatively little is known about how the Cds1 and Chk1 families respond to checkpoint signals. For example, in budding yeast, Rad53, a presumed target of Mec1, must bind to Rad9 to undergo phosphorylation and activation. Chk1 also becomes phosphorylated during checkpoint responses in various

organisms. Although it is widely assumed that this phosphorylation leads to activation of Chk1, experimental proof of this possibility has not been provided. In fission yeast, both the phosphorylation of Chk1 and the ability of Chk1 to function in checkpoint control depend upon Crb2/Rhp9, a relative of budding yeast Rad9. Genetic and two-hybrid experiments have established a close relationship between Chk1 and Crb2/Rhp9, but a direct interaction between these proteins has not been reported.

[0009] In view of the importance of cell division and development, there is a need for determining further biochemical components of checkpoint mechanisms. This invention meets that need and provides methods of using such components.

SUMMARY OF THE INVENTION

[0010] The present invention provides a family of proteins involved in regulating progression of the cell cycle. Specifically, the present invention is based on the discovery that a novel family of proteins, the Claspin proteins, plays a significant role in the activation of kinases important in regulating the cell cycle.

[0011] In one embodiment of the invention there is provided a substantially pure Claspin polypeptide characterized as (a) specifically interacting with a Chk1 protein; (b) having SQ/TQ motifs; (c) having an isoelectric point of about 4.5; and (d) having at least one nuclear localization signal. In an illustrative example, there is provided a substantially pure polypeptide having an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, or conservative variants.

[0012] In yet another embodiment of the invention, there is provided a substantially pure polypeptide having the contiguous amino acid sequences LAAVSDLNPNAPR (SEQ ID NO:6) or YLADGDLHSDGPGR (SEQ ID NO:7).

[0013] In still another embodiment of the invention, there is provided an isolated polynucleotide. The polynucleotide can be (a) a polynucleotide encoding a polypeptide having

an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4; (b) a polynucleotide of (a), where T can be U; (c) a polynucleotide complementary to (a) or (b); (d) a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3; or (d) degenerate variants of (a), (b), (c) or (d). The polynucleotide can be DNA or RNA. Also included in the present invention are isolated nucleotide fragments that hybridize to the polynucleotides of (a), (b), (c), or (d) and having as least 15 continuous bases.

[0014] Also included in the present invention is a method for identifying a compound that modulates cell cycle progression. The method includes incubating a compound and a cell expressing a Claspin protein and a chk1 protein under conditions sufficient to permit the compound to interact with the components. The cell cycle progression in a cell incubated with the compound is compared with the cell cycle progression of a cell not incubated with the compound. A difference in progression through the cell cycle between the cells is indicative of a compound that modulates cell cycle progression.

[0015] Also provided by the present invention is a method for modulating cell cycle progression in a cell providing to the cell a reagent that affects the activity or expression of a Claspin polypeptide, thereby modulating cell cycle progression.

[0016] In yet another embodiment of the invention, there is provided a method for modulating cell cycle progression in a cell. The method comprises providing to the cell a reagent that modulates the activity or expression of a chk1 polypeptide, thereby modulating the cell cycle progression. The chk1 polypeptide is human Chk1 and the reagent is a human Claspin polypeptide.

[0017] In still another embodiment of the invention there is provided a method of treating a subject having a disorder associated with increased cell cycle progression as compared to a subject not having a cell cycle disorder. The method includes administering to a subject in need of treatment a therapeutically effective amount of a compound that increases a Claspin polypeptide activity, thereby treating the disorder.

[0018] In yet another embodiment of the invention, there is provided a method of treating a subject having a disorder associated with a Claspin-associated protein activity. The method includes administering to a subject in need thereof a therapeutically effective amount of a reagent that modulates Claspin activity.

[0019] In a further embodiment of the invention, there is provided a method of diagnosing a disorder associated with cell cycle progression in a subject. The method includes determining the level of Claspin mRNA or protein expression in a sample obtained from the subject. A low level of claspin mRNA or protein expression in the subject compared to the level in a subject not having a claspin-associated disorder is indicative of a disorder associated with cell cycle progression.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] **Figures 1A** and **1B** show the nucleotide sequence of Xenopus Claspin (SEQ ID NO:1; Accession Number AF297867). **Figure 1C** shows the amino acid sequence of Xenopus Claspin (SEQ ID NO:2).

[0021] **Figure 2A** and **2B** show the nucleotide sequence of human Claspin (SEQ ID NO:3; Accession Number AF297866). **Figure 2C** shows the amino acid sequence of human Claspin (SEQ ID NO:4)

[0022] **Figure 3** is a flow diagram illustrating a computer system, data retrieving device and display.

[0023] **Figure 4** is a flow diagram illustrating one embodiment of process 200 for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database.

[0024] **Figure 5** is a flow diagram illustrating one embodiment of a process 250 in a computer for determining whether two sequences are homologous.

[0025] **Figure 6** is a flow diagram illustrating one embodiment of a process 300 for comparing features in polynucleotide and polypeptide sequences.

[0026] **Figures 7A-V** show the genomic sequence of human Claspin (SEQ ID NO:5).

DETAILED DESCRIPTION OF THE INVENTION

[0027] Checkpoint mechanisms during the cell cycle, critically important for appropriate cellular development, involve numerous components. In the absence of a key component, cell division can proceed despite incomplete or erroneous DNA replication. One novel component, identified through phylogeny, is Claspin, a protein that is required for the activation of another key component, Chk1, during a replication checkpoint mechanism. Claspin binds to Chk1 allowing Chk1 to be phosphorylated and activated, and providing an arrest of the cell cycle.

[0028] Accordingly, in one embodiment of the invention, there is provided a substantially pure Claspin polypeptide characterized as specifically interacting with a Chk1 protein, as having SQ/TQ motifs, an isoelectric point of about 4.5 and at least one nuclear localization signal Claspin polypeptides specifically interact with a chk1 protein. For example, *Xenopus* Claspin polypeptide interacts with Xchk1, a Xenopus chk1. Xenopus Chk1 (Xchk1) is required for the checkpoint-associated delay of the cell cycle in frog egg extracts containing unreplicated or DNA or DNA damaged by UV radiation. Mammalian homologues of Xchk1 have been identified and Chk1 proteins share similar biochemical functions. Chk1 proteins are effector kinases that receive signals from checkpoint sensors located upstream of Chk1 in the response pathway to DNA damage. Chk1 proteins phosphorylate and inhibit the function of Cdc25, the protein phosphatase that dephosphorylates tyrosine-15 of the cyclin-dependent kinase (cdk) Cdc2.

[0029] Claspin polypeptides have SQ/TQ motifs. Xenopus and mammalian Claspin contain a relatively large number of SQ/TQ motifs. The *Xenopus* protein has eight SQ and four TQ motifs, and human Claspin contains nine SQ and three TQ motifs. Serine (S) and threonine (T) residues, each adjacent to a glutamine (Q) residue in this type of motif, are potential substrates for kinases

such as ATM, ATR, and DNA-PK that are involved in checkpoint pathways (Kim *et al.*, (1999) *J. Biol. Chem. 274*, 37538-37543).

[0030] Claspin polypeptides are further characterized by having an isoelectric point of about 4.5. The proteins are acidic, and therefore may exhibit anomalous migration, *i.e.,* migration inconsistent with the actual molecular mass of the protein when subjected to a gel electrophoretic technique, for example, SDS-PAGE.

[0031] Claspin polypeptides have at least one nuclear localization signal. *Xenopus* Claspin and human Claspin have three conserved nuclear localization signals. In Xenopus Claspin (SEQ ID NO:2) the nuclear localization signal sites are located at amino acids 158-174, 312-316, and 1078-1084. In Human Claspin (SEQ ID NO:4) the nuclear localization signal sites are located at amino acids 152-170, 319-323 and 1132-1138. A nuclear localization signal is a motif involved in importing cytosolic proteins into the nucleus. There is no apparent conservation of sequence in nuclear localization signals amongst different protein families, although the shape and a predominance of basic amino acids are important features. The motifs are generally short in length, may contain a proline residue to break alpha-helix formation upstream of the basic residues. Hydrophobic residues are not usually included in the motif (*see* Genes VII, *ed.* B. Lewin, Oxford University Press, 2000).

[0032] Exemplary Claspin polypeptides are set forth in SEQ ID NO:2, SEQ ID NO:4 and conservative variants thereof. The terms "conservative variation" and "substantially similar" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like. The terms "conservative variation" and "substantially similar" also include the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

[0033] In one embodiment of the invention, the polypeptide is identical with or homologous to a Claspin polypeptide, such as a mammalian Claspin represented by SEQ ID NO:4, or a Xenopus Claspin represented by SEQ ID NO:2. For instance, the Claspin polypeptide preferably has an amino acid sequence at least 60% homologous to a polypeptide represented by SEQ ID Nos:2 or 4, although polypeptides with higher sequence homologies of, for example, 70%, 80%, 90% or 95% are also included in the present invention. The Claspin polypeptides can comprise a full length protein, such as represented in the sequence listings, or it can comprise a fragment of, for instance, at least 5, 10, 20, 50, 100, 150 or 200 amino acids in length.

[0034] As is well known, genes encoding a particular polypeptide may exist in single or multiple copies within the genome of an individual. Such duplicate genes may be identical or may have certain modifications, including nucleotide substitutions, additions or deletions, which all still code for polypeptides having substantially the same activity. The term "nucleic acid sequence encoding a Claspin polypeptide" may thus refer to one or more genes within a particular individual. Moreover, individual organisms may bear different nucleotide sequences, called alleles, which code for substantially the same polypeptide. Such allelic differences may or may not result in differences in amino acid sequence of the encoded polypeptide yet still encode a protein with the same biological activity.

[0035] Also provided by the invention is a substantially pure polypeptide having the contiguous amino acid sequences LAAVSDLNPNAPR (SEQ ID NO:6) or YLADGDLHSDGPGR (SEQ ID NO:7).

[0036] "Homology" refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An "unrelated" or "non-homologous" sequence shares less than 40 percent

identity, though preferably less than 25 percent identity, with one of the Claspin sequences of the present invention.

[0037] The term "isolated" as also used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs, or RNAs, respectively, that are present in the natural source of the macromolecule. For example, an isolated nucleic acid encoding one of the subject Claspin polypeptides preferably includes no more than 10 kilobases (kb) of nucleic acid sequence which naturally immediately flanks the Claspin gene in genomic DNA, more preferably no more than 5 kb of such naturally occurring flanking sequences, and most preferably less than 1.5 kb of such naturally occurring flanking sequence. The term isolated as used herein also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an "isolated nucleic acid" is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state.

[0038] Exemplary polynucleotides encoding Claspin protein are set forth in SEQ ID NO:1 and SEQ ID NO:3. The term "polynucleotide", "nucleic acid", "nucleic acid sequence", or "nucleic acid molecule" refers to a polymeric form of nucleotides at least 10 bases in length. As used herein, "isolated polynucleotide" refers to a polynucleotide that is not immediately contiguous with both of the coding sequences in the naturally occurring genome of the organism from which it is derived with which it is immediately contiguous (one on the 5' end and one on the 3' end. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, a cDNA or genomic DNA) independent of other sequences. It also includes cDNA, RNA, antisense nucleic acid, and nucleic acid sequences complementary to invention polynucleotides. It also includes genomic DNA which refers to a contiguous sequence of nucleotide that includes one or more protein coding regions, introns, upstream and downstream regulatory sequences, *i.e.*, non-coding 5'- and

3'- regulatory sequences. Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide that includes coding sequence for the polypeptide as well as a polynucleotide that includes additional coding and/or non-coding sequence.

[0039] Exemplary polynucleotides encoding a polypeptide include the nucleotide sequences set forth in SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5. For example, a nucleic acid sequence encoding a human Claspin genomic sequence includes a region encoding human claspin protein (nucleotides 4933 to 53909) as well as regions encoding regulatory sequences (nucleotides 1-4932), regions encoding one or more start sites (nucleotides 4933-10537), regions encoding introns, and regions encoding exons. Nucleotides located at intron-exon and exon-intron borders are potential splice sites. Such intron-exon and exon-intron sites include exon-intron 4956-4957; intron-exon 9476-9477; exon-intron 9585-9586; intron-exon 10088-10089; exon-intron 10537-10538; intron-exon 11481-11482; exon-intron 11647-11648; intron-exon 12325-12326; exon-intron 12399-12400; intron-exon 12732-12733; exon-intron 12805-12806; intron-exon 13598-13599; exon-intron 13707-13708; intron-exon 13886-13887; exon-intron 14461-14462; intron-exon 20866-20867; exon-intron 21058-21059; intron-exon 23296-23297; exon-intron 23553-23554; intron-exon 24991-24992; exon-intron 25170-25171; intron-exon 25271-25272; exon-intron 25336-25337; intron-exon 26198-26199; exon-intron 26398-26399; intron-exon 26741-26742; exon-intron 26896-26897; intron-exon 27810-27811; exon-intron 28037-28038; intron-exon 29240-29241; exon-intron 29352-29353; intron-exon 31272-31273; exon-intron 31348-31349; intron-exon 31561-31562; exon-intron 31662-31663; intron-exon 35273-35274; exon-intron 35438-35439; intron-exon 35545-35546; exon-intron 35667-35668; intron-exon 36220-36221; exon-intron 36348-36349; intron-exon 36705-36706; exon-intron 36811-36812; intron-exon 37381-37382; exon-intron 37464-37465; and intron-exon 37727-37728.

[0040] The nucleotides of the invention can be deoxyribonucleotides, ribonucleotides in which uracil (U) is present in place of thymine (T), or modified forms of either nucleotide. The nucleotides of the invention can be complementary to the deoxynucleotides or to the

ribonucleotides. A polynucleotide encoding a Claspin protein includes "degenerate variants", sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of a polypeptide encoded by the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO:3 is functionally unchanged.

[0041] A nucleic acid molecule encoding a Claspin protein includes sequences encoding functional Claspin polypeptides as well as functional fragments thereof. As used herein, the term "functional polypeptide" refers to a polypeptide which possesses biological function or activity which is identified through a defined functional assay (*see* Examples), and which is associated with a particular biologic, morphologic, or phenotypic alteration in the cell, for example an alteration in the timing of the cell cycle. The term "functional fragments of Claspin protein" refers to fragments of a Claspin protein that retain a Claspin activity, *e.g.*, the ability to interact with Chk1 proteins, and the like. Additionally, functional Claspin fragments may act as competitive inhibitors of Claspin binding, for example, biologically functional fragments varying in size from a polypeptide fragment as small as an epitope capable of binding an antibody molecule to a large polypeptide can participate in the characteristic induction or programming of biological changes within a cell.

[0042] An alternative embodiment of the invention provides nucleotide fragments having at least 15 base pairs and that hybridizes to a polynucleotide as set forth in nucleotides 1-331, 799-903, 1232-1543, 2147-2486 and 2964-4756 of SEQ ID NO:3.

[0043] Yet another embodiment of the invention provides an isolated polynucleotide, wherein the nucleotide is at least 15 base pairs in length which hybridizes under moderately to highly stringent conditions to DNA encoding a polypeptide as set forth in SEQ ID NO:2 or SEQ ID NO:4. In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (*e.g.*, GC v. AT content), and nucleic acid type (*e.g.*, RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in

selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

[0044] An example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderately stringent conditions); and 0.1 x SSC at about 68°C (highly stringent conditions). Washing can be carried out using only one of these conditions, *e.g.*, high stringency conditions, or each of the conditions can be used, *e.g.*, for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

[0045] A polynucleotide agent can be contained in a vector, which can facilitate manipulation of the polynucleotide, including introduction of the polynucleotide into a target cell. The vector can be a cloning vector, which is useful for maintaining the polynucleotide, or can be an expression vector, which contains, in addition to the polynucleotide, regulatory elements useful for expressing the polynucleotide and, where the polynucleotide encodes a peptide, for expressing the encoded peptide in a particular cell. An expression vector can contain the expression elements necessary to achieve, for example, sustained transcription of the encoding polynucleotide, or the regulatory elements can be operatively linked to the polynucleotide prior to its being cloned into the vector.

[0046] An expression vector (or the polynucleotide) generally contains or encodes a promoter sequence, which can provide constitutive or, if desired, inducible or tissue specific or developmental stage specific expression of the encoding polynucleotide, a poly-A recognition sequence, and a ribosome recognition site or internal ribosome entry site, or other regulatory elements such as an enhancer, which can be tissue specific. The vector also can contain elements required for replication in a prokaryotic or eukaryotic host system or both, as desired. Such vectors, which include plasmid vectors and viral vectors such as bacteriophage, baculovirus, retrovirus, lentivirus, adenovirus, vaccinia virus, semliki forest virus and adeno-associated virus vectors, are well known and can be purchased from a commercial source (Promega, Madison WI; Stratagene, La Jolla CA;

GIBCO/BRL, Gaithersburg MD) or can be constructed by one skilled in the art (see, for example, *Meth. Enzymol.*, Vol. 185, Goeddel, ed. (Academic Press, Inc., 1990); Jolly, *Canc. Gene Ther.* 1:51-64, 1994; Flotte, J. *Bioenerg. Biomemb.* 25:37-42, 1993; Kirshenbaum *et al.*, *J. Clin. Invest.* 92:381-387, 1993; each of which is incorporated herein by reference).

[0047] A polynucleotide useful in a method of the invention also can be operatively linked to tissue specific regulatory element, for example, a neuron specific regulatory element, such that expression of an encoded peptide agent is restricted to neurons in an individual, or to neurons in a mixed population of cells in culture, for example, an organ culture. For example, neuronal promoters such as the myelin basic protein promoter and other neuronal-specific promotes known to those of skill in the art may be used. Muscle-regulatory elements including, for example, the muscle creatine kinase promoter (Sternberg *et al.*, *Mol. Cell. Biol.* 8:2896-2909, 1988, which is incorporated herein by reference) and the myosin light chain enhancer/promoter (Donoghue *et al.*, *Proc. Natl. Acad. Sci., USA* 88:5847-5851, 1991, which is incorporated herein by reference) are well known in the art. A variety of other promoters have been identified which are suitable for up regulating expression in cardiac tissue. Included, for example, are the cardiac I-myosin heavy chain (AMHC) promoter and the cardiac I-actin promoter. Other examples of tissue-specific regulatory elements include, tissue-specific promoters, pancreatic (insulin or elastase), and actin promoter in smooth muscle cells. Through the use of promoters, such as milk-specific promoters, recombinant retroviruses may be isolated directly from the biological fluid of the progeny.

[0048] A Claspin polynucleotide of the invention can be inserted into a vector, which can be a cloning vector or a recombinant expression vector. The term "expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of a polynucleotide, particularly, with respect to the present invention, a polynucleotide encoding all or a peptide portion of a Claspin protein. Such expression vectors contain a promoter sequence, which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector generally contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable

for use in the present invention include, but are not limited to, the T7-based expression vector for expression in bacteria (Rosenberg, *et al., Gene* 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.* 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter, which can be a T7 promoter, metallothionein I promoter, polyhedrin promoter, or other promoter as desired, particularly tissue specific promoters or inducible promoters.

[0049] Viral expression vectors can be particularly useful for introducing a polynucleotide useful in a method of the invention into a cell, particularly a cell in a subject. Viral vectors provide the advantage that they can infect host cells with relatively high efficiency and can infect specific cell types. For example, a polynucleotide encoding a Claspin protein or functional peptide portion thereof can be cloned into a baculovirus vector, which then can be used to infect an insect host cell, thereby providing a means to produce large amounts of the encoded protein or peptide portion. The viral vector also can be derived from a virus that infects cells of an organism of interest, for example, vertebrate host cells such as mammalian, avian or piscine host cells. Viral vectors can be particularly useful for introducing a polynucleotide useful in performing a method of the invention into a target cell. Viral vectors have been developed for use in particular host systems, particularly mammalian systems and include, for example, retroviral vectors, other lentivirus vectors such as those based on the human immunodeficiency virus (HIV), adenovirus vectors, adeno-associated virus vectors, herpes virus vectors, vaccinia virus vectors, and the like (see Miller and Rosman, *BioTechniques* 7:980-990, 1992; Anderson *et al.*, *Nature* 392:25-30 *Suppl.*, 1998; Verma and Somia, *Nature* 389:239-242, 1997; Wilson, *New Engl. J. Med.* 334:1185-1187 (1996), each of which is incorporated herein by reference).

[0050] When retroviruses, for example, are used for gene transfer, replication competent retroviruses theoretically can develop due to recombination of retroviral vector and viral gene sequences in the packaging cell line utilized to produce the retroviral vector. Packaging cell lines in which the production of replication competent virus by recombination has been reduced or

eliminated can be used to minimize the likelihood that a replication competent retrovirus will be produced. All retroviral vector supernatants used to infect cells are screened for replication competent virus by standard assays such as PCR and reverse transcriptase assays. Retroviral vectors allow for integration of a heterologous gene into a host cell genome, which allows for the gene to be passed to daughter cells following cell division.

[0051] A polynucleotide, which can be contained in a vector, can be introduced into a cell by any of a variety of methods known in the art (Sambrook *et al.*, Molecular Cloning: A laboratory manual (Cold Spring Harbor Laboratory Press 1989); Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, MD (1987, and supplements through 1995), each of which is incorporated herein by reference). Such methods include, for example, transfection, lipofection, microinjection, electroporation and, with viral vectors, infection; and can include the use of liposomes, microemulsions or the like, which can facilitate introduction of the polynucleotide into the cell and can protect the polynucleotide from degradation prior to its introduction into the cell. The selection of a particular method will depend, for example, on the cell into which the polynucleotide is to be introduced, as well as whether the cell is isolated in culture, or is in a tissue or organ in culture or *in situ*.

[0052] Introduction of a polynucleotide into a cell by infection with a viral vector is particularly advantageous in that it can efficiently introduce the nucleic acid molecule into a cell *ex vivo* or *in vivo* (see, for example, U.S. Patent No. 5,399,346, which is incorporated herein by reference). Moreover, viruses are very specialized and can be selected as vectors based on an ability to infect and propagate in one or a few specific cell types. Thus, their natural specificity can be used to target the nucleic acid molecule contained in the vector to specific cell types. As such, a vector based on an HIV can be used to infect T cells, a vector based on an adenovirus can be used, for example, to infect respiratory epithelial cells, a vector based on a herpesvirus can be used to infect neuronal cells, and the like. Other vectors, such as adeno-associated viruses can have greater host cell range and, therefore, can be used to infect various cell types, although viral or non-viral vectors also can

be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

[0053] A polynucleotide sequence encoding a Claspin protein can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing polynucleotides having eukaryotic or viral sequences in prokaryotes are well known in the art, as are biologically functional viral and plasmid DNA vectors capable of expression and replication in a host. Methods for constructing an expression vector containing a polynucleotide of the invention are well known, as are factors to be considered in selecting transcriptional or translational control signals, including, for example, whether the polynucleotide is to be expressed preferentially in a particular cell type or under particular conditions (see, for example, Sambrook *et al.*, *supra*, 1989).

[0054] A variety of host cell/expression vector systems can be utilized to express a Claspin polypeptide coding sequence, including, but not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors; yeast cells transformed with recombinant yeast expression vectors; plant cell systems infected with recombinant virus expression vectors such as a cauliflower mosaic virus or tobacco mosaic virus, or transformed with recombinant plasmid expression vector such as a Ti plasmid; insect cells infected with recombinant virus expression vectors such as a baculovirus; animal cell systems infected with recombinant virus expression vectors such as a retrovirus, adenovirus or vaccinia virus vector; and transformed animal cell systems genetically engineered for stable expression. Where the expressed Claspin protein is post-translationally modified, for example, by glycosylation, it can be particularly advantageous to select a host cell/expression vector system that can effect the desired modification, for example, a mammalian host cell/expression vector system.

[0055] Depending on the host cell/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, and the like can be used in the expression vector (Bitter *et al., Meth. Enzymol.* 153:516-544, 1987). For example, when cloning in bacterial systems,

inducible promoters such as pL of bacteriophage λ, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like can be used. When cloning in mammalian cell systems, promoters derived from the genome of mammalian cells, for example, a human or mouse metallothionein promoter, or from mammalian viruses, for example, a retrovirus long terminal repeat, an adenovirus late promoter or a vaccinia virus 7.5K promoter, can be used. Promoters produced by recombinant DNA or synthetic techniques can also be used to provide for transcription of the inserted GDF receptors coding sequence.

[0056] In yeast cells, a number of vectors containing constitutive or inducible promoters can be used (see Ausubel et al., *supra*, 1987, see chapter 13; Grant *et al.*, *Meth. Enzymol.* 153:516-544, 1987; Glover, DNA Cloning Vol. II (IRL Press, 1986), see chapter 3; Bitter, Meth. Enzymol. 152:673-684, 1987; see, also, The Molecular Biology of the Yeast *Saccharomyces* (Eds., Strathern *et al.*, Cold Spring Harbor Laboratory Press, 1982), Vols. I and II). A constitutive yeast promoter such as ADH or LEU2 or an inducible promoter such as GAL can be used (Rothstein, DNA Cloning Vol. II (*supra*, 1986), chapter 3). Alternatively, vectors can be used which promote integration of foreign DNA sequences into the yeast chromosome.

[0057] Eukaryotic systems, particularly mammalian expression systems, allow for proper post-translational modifications of expressed mammalian proteins. Eukaryotic cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, phosphorylation, and advantageously, plasma membrane insertion of the gene product can be used as host cells for the expression of a Claspin protein, or functional peptide portion thereof.

[0058] Mammalian cell systems which utilize recombinant viruses or viral elements to direct expression can be engineered. For example, when using adenovirus expression vectors, the Claspin polypeptide coding sequence can be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. Alternatively, the vaccinia virus 7.5K promoter can be used (Mackett *et al., Proc. Natl. Acad. Sci., USA* 79:7415-7419, 1982; Mackett *et al.*, J. *Virol.* 49:857-864, 1984; Panicali *et al., Proc. Natl. Acad. Sci., USA* 79:4927-4931, 1982). Particularly useful are bovine papilloma virus vectors, which can replicate as

extrachromosomal elements (Sarver *et al., Mol. Cell. Biol.* 1:486, 1981). Shortly after entry of this DNA into mouse cells, the plasmid replicates to about 100 to 200 copies per cell. Transcription of the inserted cDNA does not require integration of the plasmid into the host cell chromosome, thereby yielding a high level of expression. These vectors can be used for stable expression by including a selectable marker in the plasmid, such as, for example, the neo gene. Alternatively, the retroviral genome can be modified for use as a vector capable of introducing and directing the expression of the Claspin protein gene in host cells (Cone and Mulligan, *Proc. Natl. Acad. Sci., USA* 81:6349-6353, 1984). High level expression can also be achieved using inducible promoters, including, but not limited to, the metallothionein IIA promoter and heat shock promoters.

[0059] For long term, high yield production of recombinant proteins, stable expression is preferred. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with Claspin protein cDNA controlled by appropriate expression control elements such as promoter, enhancer, sequences, transcription terminators, and polyadenylation sites, and a selectable marker. The selectable marker in the recombinant plasmid can confer resistance to the selection, and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci, which, in turn can be cloned and expanded into cell lines. For example, following the introduction of foreign DNA, engineered cells can be allowed to grow for 1 to 2 days in an enriched media, and then are switched to a selective media. A number of selection systems can be used, including, but not limited to, the herpes simplex virus thymidine kinase (Wigler *et al., Cell* 11:223, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska and Szybalski, *Proc. Natl. Acad. Sci., USA* 48:2026, 1982), and adenine phosphoribosyltransferase (Lowy, *et al., Cell* 22:817, 1980) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, *et al., Proc. Natl. Acad. Sci. USA* 77:3567, 1980; O'Hare *et al., Proc. Natl. Acad. Sci., USA* 78: 1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan and Berg, *Proc. Natl. Acad. Sci., USA* 78:2072, 1981); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin *et al., J. Mol. Biol.* 150:1, 1981); and hygro, which confers resistance to hygromycin (Santerre *et al., Gene* 30:147, 1984) genes. Additional selectable

genes, including trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman and Mulligan, *Proc. Natl. Acad. Sci., USA* 85:8047, 1988); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, *Curr. Comm. Mol. Biol.* (Cold Spring Harbor Laboratory Press, 1987), also have been described.

[0060] When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors can be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the Claspin proteins of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (Gluzman, Eukaryotic Viral Vectors (Cold Spring Harbor Laboratory Press, 1982)).

[0061] The invention provides a method for producing a polypeptide encoded by the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3 or fragments thereof, including culturing the host cell under conditions suitable for the expression of the polypeptide and recovering the polypeptide from the host cell culture.

[0062] A Claspin polypeptide or a fragment thereof, can be encoded by a recombinant or non-recombinant nucleic acid molecule and expressed in a cell. Preparation of a Claspin polypeptide by recombinant methods provides several advantages. In particular, the nucleic acid sequence encoding the Claspin polypeptide can include additional nucleotide sequences encoding, for example, peptides useful for recovering the Claspin polypeptide from the host cell. A Claspin polypeptide can be recovered using well known methods, including, for example, precipitation, gel filtration, ion exchange, reverse-phase, or affinity chromatography (see, for example, Deutscher *et al.*, Guide to Protein Purification in Meth. Enzymol., Vol. 182, (Academic Press, 1990)). Such methods also can be used to purify a fragment of a Claspin polypeptide, for example, a particular binding sequence, from a cell in which it is naturally expressed.

[0063] A recombinant nucleic acid molecule encoding a Claspin polypeptide or a fragment thereof can include, for example, a protease site, which can facilitate cleavage of the Claspin polypeptide from a non-Claspin polypeptide sequence, for example, a tag peptide, secretory peptide, or the like. As such, the recombinant nucleic acid molecule also can encode a tag peptide such as a polyhistidine sequence, a FLAG peptide (Hopp *et al., Biotechnology* 6:1204 (1988)), a glutathione S-transferase polypeptide or the like, which can be bound by divalent metal ions, a specific antibody (U.S. Patent No. 5,011,912), or glutathione, respectively, thus facilitating recovery and purification of the Claspin polypeptide comprising the peptide tag. Such tag peptides also can facilitate identification of the Claspin polypeptide through stages of synthesis, chemical or enzymatic modification, linkage, or the like. Methods for purifying polypeptides comprising such tags are well known in the art and the reagents for performing such methods are commercially available.

[0064] A nucleic acid molecule encoding a Claspin polypeptide can be engineered to contain one or more restriction endonuclease recognition and cleavage sites, which can facilitate, for example, substitution of an element of the Claspin polypeptide such as the selective recognition domain or, where present, a spacer element. As such, related Claspin polypeptides can be prepared, each having a similar activity, but having specificity for different function-forming contexts. A restriction endonuclease site also can be engineered into (or out of) the sequence coding a peptide portion of the Claspin polypeptide, and can, but need not change one or more amino acids encoded by the particular sequence. Such a site can provide a simple means to identify the nucleic acid sequence, based on cleavage (or lack of cleavage) following contact with the relevant restriction endonuclease, and, where introduction of the site changes an amino acid, can further provide advantages based on the substitution.

[0065] Antibodies of the invention may bind to Claspin polypeptides provided by the invention to prevent normal interactions of Claspin proteins. Binding of antibodies to Claspin proteins can interfere with, for example, cell cycle progression. Binding of antibodies can interfere with Claspin protein binding to intracellular proteins, for example, to a Chk1 protein and the like.

[0066] The antibodies of the invention can be used in any subject in which it is desirable to administer in vitro or in vivo immunodiagnosis or immunotherapy. The antibodies of the invention

are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

[0067] The term "antibody" as used in this invention includes intact molecules as well as fragments thereof, such as Fab, F(ab')2, and Fv which are capable of binding to an epitopic determinant present in an invention polypeptide. Such antibody fragments retain some ability to selectively bind with its antigen or receptor.

[0068] Methods of making these fragments are known in the art. (See for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1988), incorporated herein by reference). Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler & Milstein, *Nature* 256:495 (1975); Coligan *et al.*, sections 2.5.1-2.6.7; and Harlow *et al.*, *Antibodies: A Laboratory Manual*, page 726 (Cold Spring Harbor Pub. 1988), which are hereby incorporated by reference. Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen/ligand, verifying the presence of antibody production by analyzing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. *See, e.g.*, Coligan *et al.*, sections 2.7.1-2.7.12

and sections 2.9.1-2.9.3; Barnes *et al.*, "Purification of Immunoglobulin G (IgG)" in *Methods In Molecular Biology*, Vol. 10, pages 79-104 (Humana Press 1992).

[0069] Antibodies that bind to an invention polypeptide can be prepared using an intact polypeptide or fragments containing small peptides of interest as the immunizing antigen. For example, it may be desirable to produce antibodies that specifically bind to the amino- or carboxyl-terminal domains of an invention polypeptide. For the preparation of polyclonal antibodies, the polypeptide or peptide used to immunize an animal is derived from translated cDNA or chemically synthesized and can be conjugated to a carrier protein, if desired. Commonly used carrier proteins which may be chemically coupled to the immunizing peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), tetanus toxoid, and the like.

[0070] Invention polyclonal or monoclonal antibodies can be further purified, for example, by binding to and elution from a matrix to which the polypeptide or a peptide to which the antibodies were raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (See, for example, Coligan, *et al.*, Unit 9, Current Protocols in Immunology, Wiley Interscience, 1994, incorporated herein by reference).

[0071] The antibodies of the invention can be bound to many different carriers and used to detect the presence of an antigen comprising the polypeptides of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention . Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

[0072] There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds,

phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

[0073] Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific antihapten antibodies.

[0074] In using the monoclonal and polyclonal antibodies of the invention for the in vivo detection of antigen, *e.g.,* a Claspin protein, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled antibody is administered in sufficient quantity to enable detection of the site having the antigen comprising a polypeptide of the invention for which the antibodies are specific.

[0075] The concentration of detectably labeled antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. Further, it is desirable that the detectably labeled antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

[0076] As a rule, the dosage of detectably labeled antibody for in vivo treatment or diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

[0077] In another series of embodiments, the present invention provides transgenic animal models diseases or disorders associated with mutations in the Claspin protein genes. The animal may be essentially any amphibian, reptile, fish, mammal, and the like. Preferably, the transgenic animal is mammalian including rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and non-human primates. In addition, invertebrate models, including nematodes and insects,

may be used for certain applications. The animal models are produced by standard transgenic methods including microinjection, transfection, or by other forms of transformation of embryonic stem cells, zygotes, gametes, and germ line cells with vectors including genomic or cDNA fragments, minigenes, homologous recombination vectors, viral insertion vectors and the like. Suitable vectors include vaccinia virus, adenovirus, adeno associated virus, retrovirus, liposome transport, neurotropic viruses, herpes simplex virus, and the like. The animal models may include transgenic sequences comprising or derived from Claspin proteins including normal and mutant sequences, intronic, exonic and untranslated sequences, and sequences encoding subsets of Claspin proteins such as functional domains.

[0078] The major types of animal models provided include: (1) Animals in which a normal Claspin gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; in which a normal Claspin gene has been recombinantly substituted for one or both copies of the animal's homologous Claspin gene by homologous recombination or gene targeting; and/or in which one or both copies of one of the animal's homologous Claspin genes have been recombinantly "humanized" by the partial substitution of sequences encoding the human homologue by homologous recombination or gene targeting. (2) Animals in which a mutant Claspin gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; in which a mutant Claspin gene has been recombinantly substituted for one or both copies of the animal's homologous Claspin gene by homologous recombination or gene targeting; and/or in which one or both copies of one of the animal's homologous Claspin genes have been recombinantly "humanized" by the partial substitution of sequences encoding a mutant human homologue by homologous recombination or gene targeting. (3) Animals in which a mutant version of one of that animal's Claspin genes has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene or a large genomic fragment; and/or in which a mutant version of one of that animal's claspin genes has

been recombinantly substituted for one or both copies of the animal's homologous Claspin gene by homologous recombination or gene targeting. (4) "Knock-out" animals in which one or both copies of one of the animal's Claspin genes have been partially or completely deleted by homologous recombination or gene targeting, or have been inactivated by the insertion or substitution by homologous recombination or gene targeting of exogenous sequences.

[0079] In a preferred embodiment of the invention, there is provided a transgenic non-human animal having a transgene that expresses a Claspin-encoding polynucleotide chromosomally integrated into the germ cells of the animal. Animals are referred to as "transgenic" when such animal has had a heterologous DNA sequence, or one or more additional DNA sequences normally endogenous to the animal (collectively referred to herein as "transgenes") chromosomally integrated into the germ cells of the animal. The transgenic animal (including its progeny) will also have the transgene fortuitously integrated into the chromosomes of somatic cells.

[0080] Various methods to make the transgenic animals of the subject invention can be employed. Generally speaking, three such methods may be employed. In one such method, an embryo at the pronuclear stage (a "one cell embryo") is harvested from a female and the transgene is microinjected into the embryo, in which case the transgene will be chromosomally integrated into both the germ cells and somatic cells of the resulting mature animal. In another such method, embryonic stem cells are isolated and the transgene incorporated therein by electroporation, plasmid transfection or microinjection, followed by reintroduction of the stem cells into the embryo where they colonize and contribute to the germ line. Methods for microinjection of mammalian species is described in United States Patent No. 4,873,191. In yet another such method, embryonic cells are infected with a retrovirus containing the transgene whereby the germ cells of the embryo have the transgene chromosomally integrated therein. When the animals to be made transgenic are avian, because avian fertilized ova generally go through cell division for the first twenty hours in the oviduct, microinjection into the pronucleus of the fertilized egg is problematic due to the inaccessibility of the pronucleus. Therefore, of the methods to make transgenic animals described generally above, retrovirus infection is preferred for avian species, for example as described in U.S.

Patent No. 5,162,215. If microinjection is to be used with avian species, however, a recently published procedure by Love *et al.*, (Biotechnology, 12, Jan 1994) can be utilized whereby the embryo is obtained from a sacrificed hen approximately two and one-half h after the laying of the previous laid egg, the transgene is microinjected into the cytoplasm of the germinal disc and the embryo is cultured in a host shell until maturity. When the animals to be made transgenic are bovine or porcine, microinjection can be hampered by the opacity of the ova thereby making the nuclei difficult to identify by traditional differential interference-contrast microscopy. To overcome this problem, the ova can first be centrifuged to segregate the pronuclei for better visualization.

[0081] The non-human animals of the invention are murine typically (*e.g.*, mouse). The transgenic non-human animals of the invention are produced by introducing "transgenes" into the germline of the non-human animal. Embryonal target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonal target cell. The zygote is the best target for microinjection. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host gene before the first cleavage (Brinster *et al., Proc. Natl. Acad. Sci. USA* 82:4438-4442, 1985). As a consequence, all cells of the transgenic non-human animal will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene.

[0082] The term "transgenic" is used to describe an animal which includes exogenous genetic material within all of its cells. A "transgenic" animal can be produced by cross-breeding two chimeric animals which include exogenous genetic material within cells used in reproduction. Twenty-five percent of the resulting offspring will be transgenic *i.e.*, animals which include the exogenous genetic material within all of their cells in both alleles. 50% of the resulting animals will include the exogenous genetic material within one allele and 25% will include no exogenous genetic material.

[0083] In the microinjection method useful in the practice of the subject invention, the transgene is digested and purified free from any vector DNA *e.g.* by gel electrophoresis. It is preferred that the

transgene include an operatively associated promoter which interacts with cellular proteins involved in transcription, ultimately resulting in constitutive expression. Promoters useful in this regard include those from cytomegalovirus (CMV), Moloney leukemia virus (MLV), and herpes virus, as well as those from the genes encoding metallothionine, skeletal actin, P-enolpyruvate carboxylase (PEPCK), phosphoglycerate (PGK), DHFR, and thymidine kinase. Promoters for viral long terminal repeats (LTRs) such as Rous Sarcoma Virus can also be employed. Constructs useful in plasmid transfection of embryonic stem cells will employ additional regulatory elements well known in the art such as enhancer elements to stimulate transcription, splice acceptors, termination and polyadenylation signals, and ribosome binding sites to permit translation.

[0084] Retroviral infection can also be used to introduce transgene into a non-human animal, as described above. The developing non-human embryo can be cultured in vitro to the blastocyst stage. During this time, the blastomeres can be targets for retro viral infection (Jaenich, (1976) *Proc. Natl. Acad. Sci USA* 73:1260-1264). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan, *et al.* (1986) in Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The viral vector system used to introduce the transgene is typically a replication-defective retro virus carrying the transgene (Jahner, *et al.,* (1985) *Proc. Natl. Acad. Sci. USA* 82:6927-6931; Van der Putten, *et al.,* (1985) *Proc. Natl. Acad. Sci USA* 82:6148-6152,). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten, *supra*; Stewart, *et al., (*1987) *EMBO J.* 6:383-388) . Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoel (Jahner *et al.* (1982) *Nature* 298:623-628). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic nonhuman animal. Further, the founder may contain various retro viral insertions of the transgene at different positions in the genome which generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (Jahner *et al., supra*).

[0085] A third type of target cell for transgene introduction is the embryonal stem cell (ES). ES cells are obtained from pre-implantation embryos cultured in vitro and fused with embryos (M. J. Evans *et al. Nature* 292:154-156, 1981; M.O. Bradley *et al., Nature* 309: 255-258, 1984; Gossler, *et al., Proc. Natl. Acad. Sci USA* 83: 9065-9069, 1986; and Robertson *et al., Nature* 322:445-448, 1986). Transgenes can be efficiently introduced into the ES cells by DNA transfection or by retro virus-mediated transduction. Such transformed ES cells can thereafter be combined with blastocysts from a nonhuman animal. The ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal. (For review see Jaenisch, R., *Science* 240: 1468-1474, 1988).

[0086] "Transformed" means a cell into which (or into an ancestor of which) has been introduced, by means of recombinant nucleic acid techniques, a heterologous nucleic acid molecule. "Heterologous" refers to a nucleic acid sequence that either originates from another species or is modified from either its original form or the form primarily expressed in the cell.

[0087] "Transgene" means any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism (*i.e.*, either stably integrated or as a stable extrachromosomal element) which develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (*i.e.*, foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism. Included within this definition is a transgene created by the providing of an RNA sequence which is transcribed into DNA and then incorporated into the genome. The transgenes of the invention include DNA sequences which encode Claspin polypeptide-sense and antisense polynucleotides, which may be expressed in a transgenic non-human animal. The term "transgenic" as used herein additionally includes any organism whose genome has been altered by in vitro manipulation of the early embryo or fertilized egg or by any transgenic technology to induce a specific gene knockout. As used herein, The term "transgenic" includes any transgenic technology familiar to those in the art which can produce an organism carrying an introduced transgene or one in which an endogenous gene has been rendered non-functional or "knocked out".

[0088] Various methods of amplifying target sequences can be used to prepare DNA encoding a polynucleotide or nucleotide fragment according to the sequence set forth in SEQ ID NO:3. Polymerase chain reaction (PCR) technology is used to amplify such nucleic acid sequences directly from mRNA, from cDNA, from genomic libaries or cDNA libraries. Isolated sequences encoding a human Claspin protein may also be used as templates for PCR amplification. PCR techniques require the synthesis of oligonucleotide primers complementary to the two 3' borders of the DNA region to be amplified. The polymerase chain reaction is then performed using two primers. (see PCR Protocols: A Guide to Methods and Applications, Innis, Gelfand, Sninsky, and White, *eds.*, Academic Press, San Diego (1990).) Primers can be selected to amplify the entire region or regions that encode the full-length sequence of interest or to amplify smaller DNA segments.

[0089] Various method of screening and detecting nucleic acid mutations and polymorphisms are known in the art including hybridization with allele-specific oligonucleotide probes, including immobilized oligonucleotides and oligonucleotide arrays, allele-specific PCR (Newton *et al.* (1989) *Nucl. Acids, Res.* 17:2503-2516), mismatch-repair detection (Faham and Cox (1995) *Genome Res.* 5:474-482); restriction fragment length polymorphism detection based on allele-specific restriction endonuclease cleavage (Kan and Dozy (1978) *Lancet* 2: 910-912), hybridization with allele-specific oligonucleotide probes (Wallace *et al.* (1978) *Nucl Acids Res* 6: 3543-3557), including immobilized oligonucleotides (Saiki *et al.* (1989) *Proc. Natl. Acad. Sci. U.S.A.* 86: 6230-6234) or oligonucleotide arrays (Maskos and Southern (1993) *Nucl Acids Res* 21: 2269-2270), binding of MutS protein (Wagner *et al.* (1995) *Nucl Acids Res* 23: 3944-3948, denaturing-gradient gel electrophoresis (DGGE) (Fisher and Lerman *et al.* (1983) *Proc. Natl. Acad. Sci. U.S.A.* 80: 1579-1583), single-strand-conformation-polymorphism detection (Orita *et al.* (1983) *Genomics* 5: 874-879), RNAase cleavage at mismatched base-pairs (Myers *et al.* (1985) *Science* 230: 1242), chemical (Cotton *et al* (1988). *Proc. Natl. Acad. Sci. U.S.A.* 85: 4397-4401) or enzymatic (Youil *et al.* (1995) *Proc. Natl. Acad. Sci. U.S.A.* 92: 87-91) cleavage of heteroduplex DNA, methods based on allele specific primer extension (Syvanen *et al.* (1990) *Genomics* 8: 684-692), genetic bit analysis (GBA) (Nikiforov *et al.* (1994) *Nucl Acids Res* 22:

4167-4175), the oligonucleotide-ligation assay (OLA) (Landegren *et al.* (1988) *Science* 241: 1077), the allele-specific ligation chain reaction (LCR) (Barrany (1991) Proc. *Natl. Acad. Sci. U.S.A.* 88: 189-193), gap-LCR (Abravaya *et al.* (1995) *Nucl Acids Res* 23: 675-682), and radioactive and/or fluorescent DNA sequencing using standard procedures well known in the art.

[0090] A method is provided for identifying a compound that modulates cell cycle progression. The method includes incubating the compound and a cell expressing a Claspin protein under conditions sufficient to permit the compound to interact with the cell and comparing the cell cycle progression in the cell incubated with the compound with cell cycle progression of a cell not incubated with the compounds. A suitable control includes, but is not limited to, a cell cycle progression of a cell not contacted with the compound.

[0091] Modulation of cell cycle progression may be a speeding up of the cell cycle or it may be a slowing down of the cell cycle. A slowing down of the cell cycle can include an increase in the time a cell is in a gap phase, *e.g.,* G0, the pause after mitosis, G1, the gap after mitosis before DNA synthesis starts or G2, the gap after DNA synthesis is complete and before mitosis and cell division begins. The gap phases allow for checkpoints, stopping points in the cell cycle where progress can be halted. During this time, cellular mechanisms insure that DNA is not damaged, or incompletely or incorrectly replicated. Speeding up the cell cycle could allow rounds of mitosis to proceed in a shorter period of time. This may result in damaged DNA which, following mitosis, could have serious or fatal effects for the daughter cells.

[0092] Any Claspin protein may be employed in invention methods. The Claspin protein can be an un-phosphorylated form or a phosphorylated form, wherein one or more phosphate groups are chemically linked to a Claspin protein. In certain embodiments a Claspin protein according to the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4 is used.

[0093] The cell may be any cell of interest, including but not limited to neuronal cells, glial cells, cardiac cells, bronchial cells, uterine cells, testicular cells, liver cells, renal cells, intestinal cells, cells from the thymus and spleen, placental cells, endothelial cells, endocrine cells

including thyroid, parathyroid, pituitary and the like, smooth muscle cells and skeletal muscle cells. The term "incubating" includes conditions which allow contact between the test compound and the cell of interest. "Contacting" may include in solution or in solid phase.

[0094] Compounds that modulate a cellular response can include peptides, peptidomimetics, polypeptides, pharmaceuticals, chemical compounds and biological agents, for example. Antibodies, neurotropic agents, anti-epileptic compounds and combinatorial compound libraries can also be tested using the method of the invention. One class of organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 Daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups.

[0095] The test agent may also be a combinatorial library for screening a plurality of compounds. Compounds such as peptides identified in the method of the invention can be further cloned, sequenced, and the like, either in solution of after binding to a solid support, by any method usually applied to the isolation of a specific DNA sequence Molecular techniques for DNA analysis (Landegren *et al.*, *Science* 242:229-237, 1988) and cloning have been reviewed (Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Plainview, NY, 1998, herein incorporated by reference).

[0096] Candidate compounds are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to

produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc., to produce structural analogs. Candidate agents are also found among biomolecules including, but not limited to: peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[0097] A variety of other agents may be included in the screening/identification assay. These include agents like salts, neutral proteins, *e.g.*, albumin, detergents, *etc.* that are used to facilitate optimal protein-protein binding and/or reduce nonspecific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, antimicrobial agents and the like may be used. The mixture of components are added in any order that provides for the requisite binding. Incubations are performed at any suitable temperature, typically between 4 and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 10 h will be sufficient.

[0098] In another aspect of the invention, there is provided a method of modulating cell cycle progression by providing to the cell a reagent that affects the activity or expression of a Claspin polypeptide.

[0099] Claspin expression and activity are required for the proper operation of the DNA replication checkpoint in the cell cycle. Phosphorylation and activation of Chk1 proteins, preferably Xchk1, is dependent on Claspin activity. Detection of altered (decreased or increased) levels of Claspin protein activity can be accomplished by performing assays to assess cell cycle progression and, phosphorylation and activation of Chk1 proteins (see Examples section). Detection of altered (decrease or increased) levels of Claspin expression can be accomplished by numerous methods, *e.g.,* hybridization of nucleic acids isolated from a cell of interest with a Claspin polynucleotide of the invention. Analyses, such as Northern Blot analysis, are utilized to measure expression of Claspin, by assessing the level of Claspin transcript. Other standard nucleic acid detection techniques will be known to those of skill in the

art. Detection of altered levels of Claspin can also accomplished using assays designed to detect Claspin polypeptide. For example, antibodies or peptides that specifically bind a Claspin polypeptide can be utilized. Analyses, such as a radioimmune assay or an enzyme-linked immunosorbant assay (ELISA) are then used to measure Claspin, such as to measure protein concentration qualitatively or quantitatively. Immunohistochemical methods can also be used to assess expression of Claspin protein.

[0100] The term "modulating the cell cycle progression" refers to altering the cell cycle by inhibiting its progress or by stimulating its progress. For example, inhibition of the cell cycle can be accomplished by inducing a gap phase that would not have occurred in the absence of modulation or increasing the duration of a gap phase relative to the duration in the absence of modulation. Stimulation of the cell cycle can be accomplished by eliminating a gap phase that would have occurred in the absence of modulation or decreasing the duration of a gap phase relative to the duration in the absence of modulation.

[0101] The term "reagent" as used herein describes any molecule, *e.g.*, protein, nucleic acid, or pharmaceutical, with the capability of altering the expression of Claspin polynucleotide or activity of Claspin polypeptide. Candidate reagents include nucleic acids encoding a Claspin polypeptide, or that interfere with expression of a Claspin polypeptide, such as an antisense nucleic acid, ribozymes, and the like. Candidate reagents further include antibodies that specifically recognize Claspin polypeptides. Candidate reagents also encompass numerous chemical classes wherein the agent modulates Claspin expression or activity. One exemplary reagent is caffeine.

[0102] In another embodiment of the invention, there is provided a method for modulating a cell cycle progression. The method includes providing to the cell a reagent that modulates the activity or expression of a Chk1 polypeptide. The Chk1 polypeptide can be, for example, human Chk1, and the reagent can be a human Claspin polypeptide. One exemplary human Claspin polypeptide has the amino acid sequence set forth in SEQ ID NO:4.

[0103] In still another embodiment of the invention, there is provided a method for identifying a compound that can block the interaction of a Claspin protein with a Chk1 protein. The method includes incubating a candidate compound with a cell expressing a Claspin protein and a Chk1 protein under conditions that allow the compound to interact with the cell. The interaction of the Claspin protein with the Chk1 protein is compared to the interaction of the Claspin protein with a Chk1 protein in a cell not incubated with the compound. A difference in interaction, in particular, an interaction is completely or partially blocked, is indicative of a compound that can block the interaction of a Claspin protein with a Chk1 protein. In another embodiment, the method includes incubating the candidate compound in an egg extract with a Claspin protein and a Chk1 protein under conditions that allow the compound to interact with the Claspin protein or the Chk1 protein. A difference in interaction, i.e., binding of Claspin to Chk1 between the extract containing the compound and an extract without the compound is indicative of a compound that can block the interaction of a Claspin protein with a Chk1 protein.

[0104] In yet another embodiment of the invention, there is provided a method of treating a subject having a disorder associated with cell cycle progression. The method includes administering to a subject in need thereof a therapeutically effective amount of a compound that modulates a Claspin polypeptide activity.

[0105] A method is further provided for treating a disorder associated with a Claspin protein activity. The method includes administering to a subject in need thereof a therapeutically effective amount of a reagent that modulates a Claspin protein activity.

[0106] Treatment of a disorder associated with cell cycle progression may influence Claspin binding to Chk1 proteins, Claspin binding to kinases or phosphatases, phosphorylation of a Chk1 protein, cell number, rounds of mitosis, DNA repair, functional properties of Claspin proteins, and the like.

[0107] Modulation of a Claspin polypeptide activity envisions the suppression of Claspin activity or expression when Claspin is overexpressed or has an increased activity as compared to

a control. The term “modulate” also includes the augmentation of the expression of Claspin polypeptide when it is underexpressed or has a decreased activity as compared to a control.

[0108] The disorder can be a cell proliferative disorder. A cell proliferative disorder as described herein may be a neoplasm. Such neoplasms are either benign or malignant. The term "neoplasm" refers to a new, abnormal growth of cells or a growth of abnormal cells that reproduce faster than normal. A neoplasm creates an unstructured mass (a tumor) which can be either benign or malignant. The term "benign" refers to a tumor that is noncancerous, e.g. its cells do not invade surrounding tissues or metastasize to distant sites. The term "malignant" refers to a tumor that is metastastic, invades contiguous tissue or no longer under normal cellular growth control. Neoplasms are generally derived from cells that normally maintain a proliferative capacity; almost every cell and tissue type can give rise to a neoplasm.

[0109] Where a disorder is associated with the increased expression of a Claspin polypeptide, nucleic acid sequences that interfere with the expression of a Claspin polypeptide can be used (see Kushner and Silverman (2000) *Curr. Oncol. Reports*, 2:23-30, herein incorporated by reference). In this manner, binding to a Chk1 protein, phosphorylation of a Chk1 protein, cell cycle progression, and the like can be modulated. This approach also utilizes, for example, antisense nucleic acid, ribozymes, or triplex agents to block transcription or translation of a Claspin mRNA, either by masking that mRNA with an antisense nucleic acid or triplex agent, or by cleaving it with a ribozyme in disorders associated with increased Claspin expression. Alternatively, a dominant negative form of a Claspin polypeptide could be administered.

[0110] When Claspin is overexpressed, candidate agents include antisense nucleic acid sequences. Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub (1990) *Scientific American*, 262:40). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause

problems than larger molecules when introduced into the target cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, (1988) *Anal. Biochem.*, 172:289).

[0111] Use of an oligonucleotide to stall transcription is known as the triplex strategy since the oligomer winds around double-helical DNA, forming a three-strand helix. Therefore, these triplex compounds can be designed to recognize a unique site on a chosen gene (Maher *et al.*, (1991) *Antisense Res. and Dev.*, 1:227; Helene, (1991) *Anticancer Drug Design*, 6:569).

[0112] Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, 1988, *J. Amer. Med. Assn.*, 260:3030). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

[0113] There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff (1988) *Nature*, 334:585) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

[0114] When a disorder is associated with the decreased expression of a Claspin polypeptide, nucleic acid sequences that encode a Claspin polypeptide can be used. An agent which modulates claspin expression includes a polynucleotide encoding a polypeptide of SEQ ID NO:2, SEQ ID NO:4, or a conservative variant thereof. Alternatively, an agent of use with the subject invention includes reagents that increase the expression of a polynucleotide encoding Claspin or a reagent that increases the activity of a Claspin polypeptide. Another embodiment of

the invention provides a computer readable medium having store thereon a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, and sequences substantially identical thereto, or a polypeptide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, and sequences substantially identical thereto.

[0115] In another embodiment of the invention, there is provided a kit for diagnosing a claspin-associated disorder. The kit includes a nucleic acid composition for measuring in a cell sample from a subject a level of nucleic acid encoding a human Claspin.

[0116] A further embodiment of the invention provides a computer system comprising a processor and a data storage device wherein said date storage device has stored thereon a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, and sequences substantially identical thereto, or a polypeptide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, and sequences substantially identical thereto. The computer system, additionally can contain a sequence comparison algorithm and a data storage device having at least one reference sequence stored on it. The sequence comparison algorithm comprises a computer program which indicates polymorphisms. The term "polymorphism", as used herein, refers to the existence of multiple alleles at a single locus. Polymorphism can be are several types including, for example, those that change DNA sequence but do not change protein sequence, those that change protein sequence without changing function, those that create proteins with a different activity, and those that create proteins that are non-functional.

[0117] Embodiments of the invention include systems (*e.g.*, internet based systems), particularly computer systems which store and manipulate the coordinate and sequence information described herein. One example of a computer system 100 is illustrated in block diagram form in Figure 3. As used herein, "a computer system" refers to the hardware components, software components, and data storage components used to analyze invention sequences. The computer system 100 typically includes a processor for processing, accessing and manipulating the sequence data. The processor 105 can be any well-known type of central

processing unit, such as, for example, the Pentium III from Intel Corporation, or similar processor from Sun, Motorola, Compaq, AMD or International Business Machines.

[0118] Typically the computer system 100 is a general purpose system that comprises the processor 105 and one or more internal data storage components 110 for storing data, and one or more data retrieving devices for retrieving the data stored on the data storage components. A skilled artisan can readily appreciate that any one of the currently available computer systems are suitable.

[0119] In one particular embodiment, the computer system 100 includes a processor 105 connected to a bus which is connected to a main memory 115 (preferably implemented as RAM) and one or more internal data storage devices 110, such as a hard drive and/or other computer readable media having data recorded thereon. In some embodiments, the computer system 100 further includes one or more data retrieving device 118 for reading the data stored on the internal data storage devices 110.

[0120] The data retrieving device 118 may represent, for example, a floppy disk drive, a compact disk drive, a magnetic tape drive, or a modem capable of connection to a remote data storage system (*e.g.*, via the internet) etc. In some embodiments, the internal data storage device 110 is a removable computer readable medium such as a floppy disk, a compact disk, a magnetic tape, etc. containing control logic and/or data recorded thereon. The computer system 100 may advantageously include or be programmed by appropriate software for reading the control logic and/or the data from the data storage component once inserted in the data retrieving device.

[0121] The computer system 100 includes a display 120 which is used to display output to a computer user. It should also be noted that the computer system 100 can be linked to other computer systems 125a-c in a network or wide area network to provide centralized access to the computer system 100.

[0122] Figure 4 is a flow diagram illustrating one embodiment of a process 200 for comparing a new nucleotide or protein sequence with a database of sequences in order to

determine the homology levels between the new sequence and the sequences in the database. The database of sequences can be a private database stored within the computer system 100, or a public database such as GENBANK that is available through the Internet.

[0123] The process 200 begins at a start state 201 and then moves to a state 202 wherein the new sequence to be compared is stored to a memory in a computer system 100. As discussed above, the memory could be any type of memory, including RAM or an internal storage device.

[0124] The process 200 then moves to a state 204 wherein a database of sequences is opened for analysis and comparison. The process 200 then moves to a state 206 wherein the first sequence stored in the database is read into a memory on the computer. A comparison is then performed at a state 210 to determine if the first sequence is the same as the second sequence. It is important to note that this step is not limited to performing an exact comparison between the new sequence and the first sequence in the database. Well-known methods are known to those of skill in the art for comparing two nucleotide or protein sequences, even if they are not identical. For example, gaps can be introduced into one sequence in order to raise the homology level between the two tested sequences. The parameters that control whether gaps or other features are introduced into a sequence during comparison are normally entered by the user of the computer system.

[0125] Once a comparison of the two sequences has been performed at the state 210, a determination is made at a decision state 210 whether the two sequences are the same. Of course, the term "same" is not limited to sequences that are absolutely identical. Sequences that are within the homology parameters entered by the user will be marked as "same" in the process 200.

[0126] If a determination is made that the two sequences are the same, the process 200 moves to a state 214 wherein the name of the sequence from the database is displayed to the user. This state notifies the user that the sequence with the displayed name fulfills the homology constraints that were entered. Once the name of the stored sequence is displayed to the user, the process 200

moves to a decision state 218 wherein a determination is made whether more sequences exist in the database. If no more sequences exist in the database, then the process 200 terminates at an end state 220. However, if more sequences do exist in the database, then the process 200 moves to a state 224 wherein a pointer is moved to the next sequence in the database so that it can be compared to the new sequence. In this manner, the new sequence is aligned and compared with every sequence in the database.

[0127] It should be noted that if a determination had been made at the decision state 212 that the sequences were not homologous, then the process 200 would move immediately to the decision state 218 in order to determine if any other sequences were available in the database for comparison.

[0128] Figure 5 is a flow diagram illustrating one embodiment of a process 250 in a computer for determining whether two sequences are homologous. The process 250 begins at a start state 252 and then moves to a state 254 wherein a first sequence to be compared is stored to a memory. The second sequence to be compared is then stored to a memory at a state 256. The process 250 then moves to a state 260 wherein the first character in the first sequence is read and then to a state 262 wherein the first character of the second sequence is read. It should be understood that if the sequence is a nucleotide sequence, then the character would normally be either A, T, C, G or U. If the sequence is a protein sequence, then it is preferably in the single letter amino acid code so that the first and sequence sequences can be easily compared.

[0129] A determination is then made at a decision state 264 whether the two characters are the same. If they are the same, then the process 250 moves to a state 268 wherein the next characters in the first and second sequences are read. A determination is then made whether the next characters are the same. If they are, then the process 250 continues this loop until two characters are not the same. If a determination is made that the next two characters are not the same, the process 250 moves to a decision state 274 to determine whether there are any more characters either sequence to read.

[0130] If there are not any more characters to read, then the process 250 moves to a state 276 wherein the level of homology between the first and second sequences is displayed to the user. The level of homology is determined by calculating the proportion of characters between the sequences that were the same out of the total number of sequences in the first sequence. Thus, if every character in a first 100 nucleotide sequence aligned with a every character in a second sequence, the homology level would be 100%.

[0131] Homology or identity is often measured using sequence analysis software (*e.g.*, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software matches similar sequences by assigning degrees of homology to various deletions, substitutions and other modifications. The terms "homology" and "identity" in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same when compared and aligned for maximum correspondence over a comparison window or designated region as measured using any number of sequence comparison algorithms or by manual alignment and visual inspection.

[0132] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0133] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are

optimally aligned. Methods of alignment of sequence for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482, 1981, by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443, 1970, by the search for similarity method of person & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection. Other algorithms for determining homology or identity include, for example, in addition to a BLAST program (Basic Local Alignment Search Tool at the National Center for Biological Information), ALIGN, AMAS (Analysis of Multiply Aligned Sequences), AMPS (Protein Multiple Sequence Alignment), ASSET (Aligned Segment Statistical Evaluation Tool), BANDS, BESTSCOR, BIOSCAN (Biological Sequence Comparative Analysis Node), BLIMPS (BLocks IMProved Searcher), FASTA, Intervals & Points, BMB, CLUSTAL V, CLUSTAL W, CONSENSUS, LCONSENSUS, WCONSENSUS, Smith-Waterman algorithm, DARWIN, Las Vegas algorithm, FNAT (Forced Nucleotide Alignment Tool), Framealign, Framesearch, DYNAMIC, FILTER, FSAP (Fristensky Sequence Analysis Package), GAP (Global Alignment Program), GENAL, GIBBS, GenQuest, ISSC (Sensitive Sequence Comparison), LALIGN (Local Sequence Alignment), LCP (Local Content Program), MACAW (Multiple Alignment Construction & Analysis Workbench), MAP (Multiple Alignment Program), MBLKP, MBLKN, PIMA (Pattern-Induced Multi-sequence Alignment), SAGA (Sequence Alignment by Genetic Algorithm) and WHAT-IF. Such alignment programs can also be used to screen genome databases to identify polynucleotide sequences having substantially identical sequences. A number of genome databases are available, for example, a substantial portion of the human genome is available as part of the Human Genome Sequencing Project (see J. Roach, at the uniform resource locator (url) weber.u.Washington.edu/~roach/human_ genome_ progress 2.html) (Gibbs, 1995). At least twenty-one other genomes have already been sequenced, including, for example, *M. genitalium* (Fraser *et al.*, 1995), M. jannaschii (Bult *et al.*, 1996), *H. influenzae* (Fleischmann *et al.*, 1995), *E. coli* (Blattner *et al.*, 1997), and yeast (*S. cerevisiae*) (Mewes *et al.*, 1997), and *D. melanogaster* (Adams *et al.*, 2000). Significant progress has also been made in sequencing the

genomes of model organism, such as mouse, *C. elegans*, and *Arabadopsis sp.* Several databases containing genomic information annotated with some functional information are maintained by different organization, and are accessible via the internet, for example, world wide web addresses: .tigr.org/tdb; .genetics.wisc.edu; .stanford.edu/~ball; .hiv-web.lanl.gov;.ncbi.nlm.nih.gov;.ebi.ac.uk; Pasteur.fr/other/biology; and .genome.wi.mit.edu.

[0134] One example of a useful algorithm is BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, Nuc. Acids Res. 25:3389-3402, 1977, and Altschul *et al.*, J. Mol. Biol. 215:403-410, 1990, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length of 3, and expectations (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915, 1989) alignments (B) of 50, expectation (E) of 10, M=5, N= -4, and a comparison of both strands.

[0135] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, *e.g.*, Karlin & Altschul, Proc. Natl. Acad. Sci. USA 90:5873, 1993). One measure of similarity provided by BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a references sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0136] In one embodiment, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST") In particular, five specific BLAST programs are used to perform the following task: (1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence database; (2) BLASTN compares a nucleotide query sequence against a nucleotide sequence database; (3) BLASTX compares the six-frame conceptual translation products of a query nucleotide sequence (both strands) against a protein sequence database; (4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and (5) TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

[0137] The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are preferably identified (*i.e.*, aligned) by means of a scoring matrix, many of which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (Gonnet *et al., Science* 256:1443-1445, 1992; Henikoff and Henikoff, *Proteins* 17:49-61, 1993). Less preferably, the PAM or PAM250 matrices may also be used (see, *e.g.*, Schwartz and Dayhoff, eds., 1978, *Matrices for Detecting Distance Relationships: Atlas of Protein Sequence and Structure*, Washington: National

Biomedical Research Foundation). BLAST programs are accessible through the U.S. National Library of Medicine site on the world wide web, for example

[0138] The parameters used with the above algorithms may be adapted depending on the sequence length and degree of homology studied. In some embodiments, the parameters may be the default parameters used by the algorithms in the absence of instructions from the user.

[0139] The following examples are intended to illustrate but not limit the invention.

EXAMPLE 1

Tautomycin Stabilizes the Phosphorylation of Xchk1 in *Xenopus* Egg Extracts

[0140] In the presence of incompletely replicated or UV-damaged chromosomal DNA, only a small portion of the Xchk1 in whole *Xenopus* egg extracts undergoes phosphorylation (see Kumagai *et al.*, (1998) *J. Cell Biol. 142*, 1359-1569). The phosphorylated form of Xchk1 is highly enriched in the nuclear fraction of the extracts. To study the properties of phosphorylated Xchk1 more readily, a system was developed in which Xchk1 could undergo efficient phosphorylation in whole egg extracts in the presence of synthetic oligonucleotides. The establishment of this system was facilitated by the use of a phosphatase inhibitor, tautomycin, that stabilizes the phosphorylation of Xchk1 in egg extracts.

[0141] **Production of ^{35}S-Labeled Proteins** ^{35}S-Labeled full-length Claspin, Claspin-N (amino acids l-743), and Claspin-C (amino acids 776-1285) were synthesized using pBS-Claspin, pBS-Claspin-N, and pBS-Claspin-C, respectively, as templates in the TNT *in vitro* transcription/translation system (Promega) in the presence of [^{35}S]Translabel (ICN Biomedicals). ^{35}S-Labeled-Xchkl proteins were synthesized as described (Kumagai *et al.*, 1998

[0142] 3 μM tautomycin was added to egg extracts containing *Xenopus* sperm chromatin and aphidicolin, a DNA polymerase inhibitor that induces DNA replication blocks and thereby inhibits cell cycle progression. ^{35}S-Labeled Xchk1 was incubated for 30 min in interphase egg extracts containing no sperm nuclei, 3000 nuclei/μl, 3000 nuclei/μl and 100 μl/ml aphidicolin, or 3000 UV-irradiated nuclei/μl in the presence or absence of 5 mM caffeine. The extracts were dividied, and

the incubation was continued for 70 min in the absence or presence of 3 μM tautomycin. Aliquots (2 μl) were removed for SDS-PAGE and autoradiography.

[0143] Tautomycin caused a significant accumulation of phosphorylated Xchk1. Likewise, treatment with tautomycin also resulted in an increase in the amount of phosphorylated Xchk1 that appeared in response to UV-damaged sperm chromatin. Significantly, tautomycin did not have any effect on the phosphorylation of Xchk1 in extracts lacking aphidicolin or UV-damaged DNA. Furthermore, caffeine, an agent that inhibits the checkpoint-dependent phosphorylation of Xchk1 and overrides checkpoint controls in *Xenopus* egg extracts as well as other systems (see Kumagai *et al.*, 1998), blocked the tautomycin-stimulated phosphorylation of Xchk1 in extracts containing aphidicolin or UV-damaged DNA.

[0144] It is necessary to add tautomycin to egg extracts at least 30 min after the addition of *Xenopus* sperm chromatin and aphidicolin. In fact, the addition of tautomycin, sperm chromatin, and aphidicolin together at the start of the incubation actually prevents the phosphorylation of Xchk1. Removal of protein phosphatase 2A (PP2A) from egg extracts prevents the initiation of DNA replication but not elongation at existing replication forks (Lin *et al.* (1998) *Proc. Nat. Acad. Sci. USA 95*, 14693-14698). Tautomycin is an inhibitor of PP2A, among other phosphatases. Furthermore, a period of about 30 min is required for the formation of pre-replication complexes and initiation of replication in egg extracts (Coleman *et al.* (1996) Cell *87*, 53-63). Thus, premature addition of tautomycin most probably blocks the formation of DNA replication forks, which are required for activation of the DNA replication checkpoint (Li and Deshaies, (1993) Cell *74*, 223-226).

EXAMPLE 2

Phosphorylation of Xchk1 in the Presence of Synthetic Oligonucleotides

[0145] Previous studies have demonstrated that various defined DNA templates, including linearized plasmids, double-stranded oligonucleotides, partially-nicked Ml3 DNA, and poly(dT)$_{40}$, all trigger the phosphorylation of Xcdsl but not Xchk1 in *Xenopus* egg extracts (Guo and Dunphy, (2000) *Mol. Biol. Cell 11*, 1535-1546). These templates either contain double-stranded DNA ends

or undergo replication to a double-stranded form, in the case of M13 and poly(dT)$_{40}$, indicating that double-stranded DNA ends trigger the phosphorylation of Xcdsl. Nonetheless, one intriguing aspect of these studies was that, even though M13 DNA and poly(dT)$_{40}$, must be replicated to a double-stranded form to induce phosphorylation of Xcds1, these templates did not elicit the phosphorylation of Xchk1. One rationale suggests that, since these templates are not incorporated into a nuclear structure, any phosphorylated Xchk1 that could potentially be generated as a result of their presence might be more susceptible to dephosphorylation in whole egg extracts.

[0146] To test this rationale a number of different DNA templates were incubated in *Xenopus* extracts in the presence or absence of the phosphatase inhibitor tautomycin. In one set of experiments, various DNA homopolymers, *e.g.*, poly(dA)$_{70}$, poly(dT)$_{70}$, poly(dC)$_{70}$ and poly(dG)$_{70}$, were added to extracts containing ^{35}S-labeled Xchk1 and subsequently, the phosphorylation of Xchk1 was examined. ^{5}S-Labeled Xchk1 was added to egg extracts containing 50 μg/ml poly(dA)$_{70}$, 50 μg/ml poly(dT)$_{70}$, 50 μg/ml poly(dA)$_{70}$-poly(dT)$_{70}$, 50 μg/ml poly(dC)$_{70}$, 50 μg/ml poly(dG)$_{70}$, or 50 μg/ml poly(dC)$_{70}$-poly(dG)$_{70}$ in the presence or absence of 5 mM caffeine. The extracts were incubated for 100 min in the absence or presence of 3 μM tautomycin. Aliquots (2 μl)were removed for SDS-PAGE and autoradiography. pA, pT, pC, and pG refer to poly(dA)$_{70}$, poly(dT)$_{70}$, poly(dC)$_{70}$, and poly(dG)$_{70}$, respectively. In other experiments, ^{35}S-Labeled wild-type Xchkl and Xchkl-4AQ proteins were incubated for 100 min in interphase extracts containing either poly(dA)$_{70}$, or poly(dA)$_{70}$-poly(dT)$_{70}$ in the presence or absence of 5 mM caffeine. All samples contained 3 μM tautomycin.

[0147] None of these individual homopolymers had any effect on the phosphorylation of Xchk1 in the absence of tautomycin. Poly(dT)$_{70}$, induced a modest phosphorylation of Xchk1 in the presence of tautomycin. When a pre-annealed mixture of poly(dA)$_{70}$-poly(dT)$_{70}$ was added to tautomycin-containing extracts, however, there was a pronounced phosphorylation of Xchk1. Without tautomycin, this modification was significantly diminished. Pre-annealed poly(dC)$_{70}$–poly(dG)$_{70}$ was significantly less effective than poly(dA)-poly(dT)$_{70}$. There appears to be a strict requirement with regard to the length of the homopolymers. In particular, a preannealed mixture of

shorter homopolymers, such as poly(dA)$_{40}$-poly(dT)$_{40}$, was not able to induce the modification of Xchk1 efficiently.

[0148] Further characterization indicated that the phosphorylation of Xchk1 in the presence of the annealed homopolymers and tautomycin has similar properties to the modification of Xchk1 that occurs in nuclei containing unreplicated or UV-damaged DNA. For example, this phosphorylation was strongly reduced by treatment with caffeine. Furthermore, the phosphorylation that was induced by poly(dA)$_{70}$-poly(dT)$_{70}$ was largely abolished in the Xchk1-4AQ mutant. In this mutant, the phosphorylatable residues of Xchk1 in its four conserved SQ/TQ motifs (Thr-314, Ser-344, Ser-356, and Ser-365) have all been mutated to alanine. In addition, poly(dA)$_{70}$-poly(dT)$_{70}$ but not poly(dA)$_{70}$, induced a cell cycle arrest efficiently in egg extracts.

EXAMPLE 3

Production of His6-GST, Xchkl-WT-GST-His6, and Xchkl-N135A-GST-His6 Proteins in Insect Cells

[0149] A KasI-NcoI fragment encoding glutathione S-transferase (GST) was created by PCR (polymerase chain reaction) using Pfu Turbo polymerase (Stratagene) with the appropriate primers and pGEX-2T as template. The fragment was digested with KasI and NcoI and cloned into pFastBacHTa (GIBCO BRL) to yield a baculovirus vector with tandem six histidine and GST tags (pFastBacHT-GST). pFastBac encoding C-terminally tagged Xchk1 (Xchk1-WT-GST-His6) was created by the following procedure. The coding sequence of Xchkl was amplified in a PCR reaction, digested with RsrII and KasI, and cloned into pFastBacHT-GST. This plasmid was treated with BamHI and XbaI, and two annealed oligonucleotides encoding a six histidine tag were ligated into the vector to yield pFastBac-Xchkl-WT-GST-His6. pFastBac-Xchkl-N135A-GST-His6 and pFastBac-Xchk1-4AQ-GST-His6 were created by inserting the SacI-BstEII fragments of pBS-Xchkl-N135A (Kumagai *et al.*, 1998) and pBS-Xchkl-4AQ, respectively, into pFastBac-Xchkl-WT-GST-His6. The Xchkl-WT-GST-His6 protein contains a thrombin recognition sequence both between Xchkl and GST and between GST and the six histidine tag. Recombinant baculoviruses were produced using the Bac-to-Bac system (GIBCO BRL). Recombinant proteins were isolated

using nickel iminodiacetic acid (Ni-IDA) agarose as described (Kumagai and Dunphy, (1995) *Mol. Biol. Cell 6*, 199-213).

[0150] Ni-IDA beads (10 µl) containing Xchk1-WT-GST-His6 protein (5 µg) were incubated for 100 min at 23°C in 100 µl of interphase egg extract containing 3 µM tautomycin, 100 µg/ml cycloheximide, and either 50 µg/ml poly(dA)$_{70}$ or 50 µg/ml poly(dA)$_{70}$-poly(dT)$_{70}$ in the presence or absence of 5 mM caffeine. The beads were isolated by centrifugation and washed four times in 10 mM Hepes-KOH (pH 7.5) containing 150 mM NaCl, 0.5 % NP-40, 2.5 mM EGTA, and 20 mM β-glycerolphosphate. Bound proteins were eluted with 150 mM imidazole in the same buffer. The eluate was diluted to 500 µl and incubated with 10 µl Glutathione Sepharose 4 Fast Flow (Amersham Pharmacia Biotech) for 30 min at 4°C. The beads were washed three times in the same buffer and then subjected to SDS-PAGE. For protein sequencing, 30 ml of interphase egg extract and 1.5 mg of Xchkl-WT-GST-His6 protein were used to isolate Claspin. Proteins were eluted from glutathione agarose with 0.1% SDS, concentrated with a Microsep, 30K device (Filtron Technology), treated with dithiothreitol, alkylated, and subjected to SDS-PAGE. The gel was stained with SYPRO Red (Molecular Probes). Tryptic peptides from Claspin were separated on a Vydac C18 column and sequenced in the Caltech facility.

EXAMPLE 4

Identification of an Xchkl-Binding Protein

[0151] **Isolation of an Xchk1-binding protein** Nickel agarose beads (10 µl) containing 5 µg of either Xchkl-WT-GST-His6 protein or a control His6-GST protein were incubated in interphase egg extracts (100 µl) containing 3 µM tautomycin and either poly(dA)$_{70}$ or poly(dA)$_{70}$- poly(dT)$_{70}$ in the absence or presence of 5 mM caffeine. The beads were re-isolated and bound proteins were eluted with imidazole. The eluted proteins were purified further on glutathione agarose as described herein. The samples were subjected to SDS-PAGE and silver-staining. The control His6-GST protein was electrophoresed through the bottom of the gel in this experiment. Xchk1-WT-GST-His6 becomes only partially phosphorylated in the presence of poly(dA)$_{70}$-poly(dT)$_{70}$ in this case because it was added at a 25-fold molar excess over endogenous Xchkl.

[0152] Isolation of Claspin, an Xchk1-Binding Protein As one approach to understand the regulation of Xchk1, a search for proteins that bind to Xchk1 was conducted. A recombinant form of Xchk1 (Xchk1-WT-GST-His6) was incubated in *Xenopus* egg extracts under various conditions and the protein was subsequently re-isolated by sequential chromatography on nickel and glutathione agarose, respectively. A 215 kD protein was identified that had associated with Xchk1 in the presence of poly(dA)$_{70}$-poly(dT)$_{70}$. The binding of this protein was significantly reduced if either 5 mM caffeine was included in the incubation or if poly(dA))$_{70,}$, was used as the template. The 215 kD protein was not isolated from mock incubations containing no recombinant Xchk1. In these experiments, no other polypeptide was observed with an abundance comparable to that of the 215 kD protein, but additional proteins may be involved in the interaction with Xchk1.

[0153] A large-scale purification was performed to obtain a sufficient quantity of the 215 kD protein for peptide sequencing analysis. Two peptide sequences that did not belong to a previously cloned protein were identified. One combination of degenerate PCR primers based on these peptides was used to amplify a 450-bp DNA fragment, which in turn was employed to isolate a 4.8-kb *Xenopus* oocyte cDNA (GenBank accession number AF297867). This cDNA encodes a 1285 amino acid polypeptide (calculated molecular mass, 145 kD) that contains amino acid sequences (LAAVSDLNPNAPR (SEQ ID NO:6) and YLADGDLHSDGPGR (SEQ ID NO:7)) that are consistent with the peptide sequencing analysis. A BLAST search with the cDNA sequence indicated that it encodes a novel protein, which has been named Claspin.

[0154] Cloning of *Xenopus* Claspin Sets of degenerate oligonucleotides corresponding to the two peptide sequences [(L/D)AAVXDLNPNAPX (SEQ ID NO:8) and YLADGDLHSDGPGR (SEQ ID NO:7); X denotes an ambiguous amino acid] obtained from Claspin were designed. A PCR reaction with GGIGC(A/G)TTIGG(A/G)TTIA(G/A)(G/A)TCI(G/C)(A/T)IACIGCIGC (SEQ ID NO:9) and GCIGA(T/C)GGIGA(T/C)(T/C)TICA(T/C)(T/A)(G/C)IGA(T/C)GGICCIGG (SEQ ID NO:10) Ampli-Taq DNA polymerase (Perkin-Elmer), and *Xenopus* oocyte cDNA yielded a 450-bp fragment that encodes a segment of Claspin. The sequence of this fragment was used to design primers to isolate a 4.7 kb cDNA from a *Xenopus* oocyte library (Mueller *et al.*, (1995) *Mol. Biol.*

Cell 6, 119-134) with the ClonCapture kit (Clontech). A 2.3 kb ApaI-XhoI fragment encoding the N-terminal half of the protein and a 2.4 kb XhoI-XhoI fragment encoding the C-terminal half of the protein were cloned into pBluescript SK- to yield pBS-Claspin-N and pBS-Claspin-C, respectively. The XhoI-XhoI fragment of pBS-Claspin-C was cloned into pBS-Claspin-N to yield a vector encoding full-length Claspin (pBS-Claspin). Nested deletions were sequenced at the Caltech DNA Sequencing Core Facility.

EXAMPLE 5

Cloning of Human Claspin

[0155] A BLAST analysis was conducted by using the sequence of *Xenopus* Claspin to search the human EST database. Several sequences with a strong homology to *Xenopus* Claspin sequence were found. Based on these sequences, two primers (CCACGGCTAGGTGCTGATGAAGATTCC (SEQ ID NO:11) and AACAGTGCTTGGCGCTTCTGGCG (SEQ ID NO:12)) were designed to isolate cDNAs by RACE (Rapid Amplification of cDNA Ends) from a human fetal Marathon-Ready-cDNA library (Clontech).

[0156] To obtain further insight into the structure of Claspin, a human homologue (Hu-Claspin) was identified using PCR primers based on sequences related to *Xenopus* Claspin in the human EST database. cDNA fragments from a human library were isolated and used to establish the full-length cDNA sequence of Hu-Claspin (GenBank accession number AF297866). Conceptual translation of the cDNA encoding Hu-Claspin yielded a 1332 amino acid polypeptide that is 49% identical to *Xenopus* Claspin. The gene for Hu-Claspin is located at p34.1-34.3 on chromosome 1 according to the GenBank entry for its genomic sequence (AL139143). Although the *Xenopus* cDNA does not contain an in-frame termination codon upstream of the putative initiation codon, it most likely represents the full-length sequence. The human cDNA contains two in-frame stop codons upstream of an initiation codon that has a position almost identical to that of the putative start codon in *Xenopus* Claspin. Furthermore, endogenous Claspin in *Xenopus* egg extracts is just slightly smaller than recombinant His6-Claspin. It should be noted that *Xenopus* and human Claspin are quite acidic proteins (pI=4.5), which may lead to anormalous migration during SDS-PAGE.

[0157] Claspin does not appear to possess any strictly defined sequence motifs that offer insight into its biochemical function. Both the *Xenopus* and human proteins have three conserved potential nuclear localization signals (amino acids 158-174,312-316, and 1078-1084 in the *Xenopus* protein). Likewise, both proteins contain a relatively large number of SQ/TQ motifs (the *Xenopus* protein has eight SQ and four TQ motifs, while Hu-Claspin contains nine SQ and three TQ motifs). The serines and threonines in this type of motif are potential substrates for kinases such as ATM, ATR, and DNX-PK that are involved in checkpoint pathways (Kim *et al.*, (1999) *J. Biol. Chem. 274*, 37538-37543). BLAST searches of the *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* databases did not reveal any obvious homologue of Claspin. However, a PSI-BLAST search identified a weak homology to the *Drosophila* CG1326 gene product (AAF47885), which is 28% identical to *Xenopus* Claspin.

EXAMPLE 6

Phosphorylation of Claspin Is Required for Binding to Xchk1

[0158] In order to characterize the interaction of Claspin with Xchk1, ^{35}S-labeled versions of full-length Claspin, its N-terminal domain (Claspin-N, amino acids 1-743), and a C-terminal fragment (Claspin-C, amino acids 776-1285) were prepared.

[0159] **Modification of Claspin.** ^{35}S-Labeled full-length Claspin, Claspin-N, and Claspin-C were incubated for 100 min in egg extracts containing poly(dA)$_{70}$ (lane 1), poly(dA)$_{70}$-poly(dT)$_{70}$, or poly(dA)$_{70}$- poly(dT)$_{70}$ and 5 mM caffeine. Aliquots were subjected to SDS-PAGE and autoradiography. In another set of experiments, egg extracts containing ^{35}S-labeled full-length Claspin, Claspin-N, and Claspin-C and poly(dA)$_{70}$, poly(dA)$_{70}$-poly(dT)$_{70}$, or poly(dA)$_{70}$-poly(dT)$_{70}$ and 5 mM caffeine were incubated in the presence of nickel agarose containing either Xchkl-WT-GST-His6 or a control His6-GST protein. The beads were isolated, washed, and subjected to SDS-PAGE and autoradiography.

[0160] **Phosphorylated Claspin associates with Xchkl.** Claspin-GST-His6 was incubated in egg extracts containing either poly(dA)$_{70}$ or poly(dA)$_{70}$-poly(dT)$_{70}$ and 3 μM tautomycin and

subsequently re-isolated with nickel agarose. The GST-His6 tag was then removed with thrombin. Portions of some samples were incubated with PP2A in either the presence or absence of 3 μM okadaic acid. The samples were subjected to SDS-PAGE directly or assayed for binding to Xchkl. For binding assays, samples were incubated at 4°C for 30 min with nickel agarose containing either Xchkl-WT-GST-His6 or control His6-GST. After washing, the beads were subjected to SDS-PAGE. All samples were immunoblotted with anti-Claspin antibodies.

[0161] Interaction of endogenous Claspin and Xchkl in extracts containing unreplicated or UV-damaged *Xenopus* sperm chromatin. Interphase extracts containing 100 μg/ml cycloheximide and no sperm nuclei, 3000 nuclei/μl , 3000 nuclei/μl and 100 μg/ml aphidicolin, or 3000 UV-damaged nuclei/μl were incubated for 100 min at 23°C in the absence or presence of 5 mM caffeine. Extracts were immunoprecipitated with control IgG or anti-Xchkl antibodies. The immunoprecipitates were subjected to SDS-PAGE and immunoblotted with anti-Claspin antibodies.

[0162] Labeled proteins were added to egg extracts in the presence of poly(dA)$_{70}$, poly(dA)$_{70}$-poly(dT)$_{70}$, or both poly(dA)$_{70}$-poly(dT)$_{70}$, and caffeine. Full-length Claspin became modified in the presence of poly(dA)$_{70}$-poly(dT)$_{70}$, but not poly(dA)$_{70}$, and this modification was reversed by caffeine. This modification appears to be restricted largely, if not exclusively, to the C-terminal domain of Claspin, since Claspin-C, but not Claspin-N, underwent a substantial, caffeine-sensitive upshift in electrophoretic mobility in the presence of poly(dA)$_{70}$-poly(dT)$_{70}$,.

[0163] The interaction of full-length Claspin, Claspin-N, and Claspin-C with Xchk1 was examined. ^{35}S-labeled versions of these proteins were incubated in egg extracts containing Xchk1-WT-GST-His6 and subsequently re-isolated the recombinant Xchk1 with nickel agarose. Both full-length Claspin and Claspin-C, but not Claspin-N, bound to Xchk1. Moreover, this interaction occurred in the presence of poly(dA)$_{70}$-poly(dT)$_{70}$, but not poly(dA)$_{70}$, and was sensitive to caffeine.

[0164] To determine whether the modification of Claspin represented phosphorylation, the up-shifted form of Claspin was treated with PP2A. Recombinant Claspin-GST-His6 was incubated in egg extracts containing poly(dA)$_{70}$ or poly(dA)$_{70}$-poly(dT)$_{70}$. Subsequently, recombinant Claspin

was re-isolated with nickel agarose. The modified form of Claspin that had appeared in response to poly(dA)$_{70}$-poly(dT)$_{70}$ was treated with PP2A in the absence or presence of okadaic acid, an inhibitor of PP2A. The modification of Claspin was reversed substantially by PP2A, and this reversal was sensitive to okadaic acid.

[0165] The binding of Claspin to Xchk1 is important for phosphorylation. Various modified forms of Claspin without the tags (removed using thrombin) was incubated with Xchk1-WT-GST-His6 or, in some cases, a control His6-GST protein, both of which were bound to nickel agarose beads. After re-isolating and washing the beads, immunoblotting with anti-Claspin antibodies showed that only the phosphorylated form of Claspin interacted specifically with Xchk1.

[0166] The physiological significance of the binding between Claspin and Xchk1 was further explored in egg extracts containing incompletely replicated or UV-damaged nuclear DNA. Demembranated *Xenopus* sperm chromatin was added to egg extracts to allow the formation of reconstituted nuclei (see Murray, (1991) *Methods Cell Biol. 36*, 581-605). To induce the formation of DNA replication blocks in the nuclear chromatin, the DNA polymerase inhibitor aphidicolin was added to these extracts (Dasso and Newport (1990). *Cell 61*, 811-823). Alternatively, UV-treated sperm chromatin can be used as a source of damaged DNA (Kumagai *et al.*, 1998). Egg extracts were incubated either in the presence of no chromatin, chromatin and aphidicolin, or UV-damaged chromatin. In some cases, caffeine was also included in the incubation. The extracts were immunoprecipitated with anti-Xchk1 or control antibodies and the immunoprecipitates were examined by immunoblotting with anti-Claspin antibodies. The interaction between endogenous Claspin and Xchk1 was increased in the presence of aphidicolin or UV-damaged DNA. Moreover, the binding between Claspin and Xchk1 was significantly reduced in the presence of caffeine.

EXAMPLE 6

Phosphorylation of Xchk1 Results in Its Activation

[0167] Chk1 undergoes phosphorylation during a checkpoint response in various experimental systems (Walworth and Bernards, (1996) *Science 271*, 353-356.; Sanchez *et al.*(1997) *Science 277*, 1497-1501; Kumagai *et al.*, 1998), but the functional consequence of this phosphorylation has not

been established. In the case of *Xenopus* egg extracts, it had been difficult to isolate the phosphorylated form of Xchk1 efficiently to address this issue conclusively. Using the technical improvements described herein for the preparation of phosphorylated Xchk1 to investigate whether this modification affects the kinase activity of Xchk1. Recombinant Xchk1-WT-GST-His6 was added to *Xenopus* egg extracts containing tautomycin and either poly(dA)$_{70}$ or poly(dA)$_{70}$-poly(dT)$_{70}$. After a 100-min incubation, recombinant Xchk1 was reisolated with glutathione agarose and assayed kinase activity toward Cdc25 with the model substrate GST-Cdc25(254-316)-WT.

[0168] The hyperphosphorylated form of Xchk1-WT-GST-His6 that was isolated from extracts containing poly(dA)$_{70}$-poly(dT)$_{70}$ was approximately 3-fold more active than the hypophosphorylated version of the protein from extracts that contained poly(dA)$_{70}$ This increase may be an underestimate of the maximum possible degree of activation because the recovery of hyperphosphorylated Xchk1 is consistently somewhat lower than that of the hypophosphorylated form. Akinase-inactive mutant of Xchk1 (Xchk1-N135A-GST-His6) and a mutant (Xchk1-4AQ-GST-His6) that cannot undergo phosphorylation of the four conserved SQ/TQ motifs of Xchk1 was also examined. The kinase-inactive mutant of Xchk1 that was isolated from either type of extract was not able to phosphorylate the Cdc25 substrate in these assays. Also, consistent with previous results (Kumagai *et al.*, 1998), kinase-inactive Xchk1 became only partially phosphorylated in the presence of poly(dA)$_{70}$-poly(dT)$_{70}$, due to its incapacity for autophosphorylation. Finally, the Xchk1-4AQ mutant did not undergo phosphorylation or an increase in kinase activity in the presence of poly(dA)$_{70}$-poly(dT)$_{70}$, indicating that phosphorylation of the SQ/TQ domain is required for the activation of Xchk1.

[0169] The kinase activity of hyperphosphorylated Xchk1 that appeared in response to aphidicolin-induced replication blocks in nuclei that had formed around sperm chromatin in egg extracts was examined. Recombinant Xchk1-WT-GST-His6 was added to egg extracts containing 3 μM tautomycin and either no nuclei or 3000 nuclei/μl and 100 μg/ml aphidicolin. After re-isolating the added Xchk1 with glutathione agarose, Xchk1 was activated three-fold when DNA

replication blocks were present. Thus, both incompletely replicated chromatin and poly(dA)$_{70}$-poly(dT)$_{70}$ elicit a similar activation of Xchk1.

EXAMPLE 7

Immunodepletion of Claspin Blocks Phosphorylation and Activation of Xchk1

[0170] *Production of His6-Claspin, His6-Claspin-N, and Claspin-GST-His6 in Insect cells* pFastBacHT-Claspin was created by cloning the NcoI-NheI and NheI-XhoI fragments of pBS-Claspin together into pFastBacHTa, thereby generating a baculovirus vector encoding a six-histidine tagged, full-length Claspin. pFastBacHT-Claspin(1-464) encoding a six-histidine tagged N-terminal fragment of Claspin (amino acids 1-464) was created by amplifying the 1.4 kb NcoI-EcoRI fragment from pBS-Claspin-N by PCR and cloning it into pFastBacHTa that had been digested with NcoI and EcoRI. A baculovirus vector encoding Claspin with GST and six histidine tags at the C-terminal end (pFastBac-Claspin-GST-His6) was prepared as follows. pBS-Claspin was used in a PCR reaction with the appropriate primers to introduce an SpeI site at the termination codon of Claspin. The PCR product was digested with NheI and SpeI and ligated to the appropriate pFastBac fragment. Baculovirus-expressed His6-Claspin, His6-Claspin(1-464), and Claspin-GST-His6 were produced as described above.

Example 8

In Vitro Claspin-Xchk1 Binding Assays

[0171] Claspin-GST-His6 protein (5 μg) bound to 10 μl of Ni-IDA beads was incubated for 100 min in interphase extracts (100 μl) containing 100 μg/ml cycloheximide, 3 μM tautomycin, and 50 μg/ml of either poly(dA)$_{70}$ or poly(dA)$_{70}$-poly(dT)$_{70}$. The beads were isolated by centrifugation and washed twice with buffer A (10 mM Hepes-KOH [pH 7.5], 150 mM NaCl, 0.1% CHAPS, 2.5 mM EGTA, and 20 mM β-glycerolphosphate), once with Hepes-buffered saline (HBS, 10 mM Hepes-KOH [pH 7.5], 150 mM NaCl), and eluted with 150 mM imidazole in HBS. Eluted proteins were treated with 0.06 U thrombin in HBS containing 2.5 mM $CaCl_2$ for 2 hrs at 4°C during dialysis against HBS to remove imidazole. Digestion was stopped by adding 1 mM PMSF and 5 mM EGTA. Undigested protein and thrombin were removed by incubating with 10 μl Ni-IDA agarose

and 5 µl aminobenzamidine agarose for 30 min at 4°C. In some cases, recombinant Claspin from extracts containing both poly(dA)$_{70}$- poly(dT)$_{70}$ and tautomycin was treated with 0.5 U protein phosphatase 2A (Upstate Biotechnology) in the presence or absence of 3 µM okadaic acid for 30 min at 23°C. The various preparations of Claspin were then incubated with either His6-GST or Xchk1-WT-GST-His6 bound to Ni-IDA beads in buffer A containing 1 mg/ml ovalbumin for 30 min at 4°C. The beads were washed three times in buffer A and bound proteins were analyzed by immunoblotting with anti-Claspin antibodies.

[0172] Isolation and Assay of Xchkl from Egg Extracts Xchk1-WT-GST-His6, Xchkl-N135A-GST-His6, and Xchk1-4AQ-GST-His6 proteins (final concentration, 6 µg/ml) were incubated for 100 min in egg extracts (100 µl) containing 100 µg/ml cycloheximide, 3 µM tautomycin, and 50 µg/ml of either poly(dA)$_{70}$ or poly(dA)$_{70}$-poly(dT)$_{70}$. Extracts were diluted to 300 µl with buffer A and centrifuged in a 1 ml Sephadex G-25 column that had been pre-equilibrated with the same buffer to remove endogenous glutathione in the extracts. Glutathione agarose (10 µl) was incubated with the flow-through fraction for 30 min at 4°C. The glutathione beads were washed three times with buffer A, once with HBS, and incubated in kinase assays with GST-Cdc25(254-316)-WT as the substrate as described (Kumagai *et al.*, 1998).

[0173] Depletion of Claspin from Egg Extracts Interphase egg extracts (170 µl) that had been activated for 15 min at 23°C by the addition of $CaCl_2$ were mixed with 30 µg of either affinity-purified anti-Claspin antibodies or control rabbit IgG bound to 30 µl of Affiprep-protein A beads (Bio-Rad) and incubated while rocking at 4°C for 1 hr. At the end of incubation, the beads were removed by centrifugation and the immunodepletion procedure was repeated.

[0174] In order to investigate the role of Claspin in the regulation of Xchk1, endogenous Claspin was immunodepletedfrom *Xenopus* egg extracts using polyclonal antibodies that were raised against an N-terminal fragment of the protein. The level of Claspin was not diminished in a mock-depleted extract that was treated with control IgG. The endogenous concentration of Claspin in egg extracts is estimated to be approximately 33 µg/ml (170 µM). As shown by immunoblotting with anti-

Claspin antibodies, the addition of 35 μg/ml His6-Claspin to Claspin-depleted extracts restored the protein to its normal level.

[0175] The state of phosphorylation of Xchk1 under various conditions was studied by adding recombinant Xchk1-WT-GST-His6 to extracts (mock-depleted; Claspin-depleted, and Claspin-depleted containing 35μg/ml His6-Claspin) in the presence of either poly(dA)$_{70}$ or poly(dA)$_{70}$-poly(dT)$_{70}$. Recombinant Xchk1 was then re-isolated from each extract with glutathione agarose and its state of phosphorylation was examined. The checkpoint-dependent phosphorylation of Xchk1 in Claspin-depleted extracts was almost completely abolished in comparison with mock-depleted extracts. Significantly, the addition of recombinant His6-Claspin to Claspin-depleted extracts completely restored the phosphorylation of Xchk1, indicating that the defect in phosphorylation of Xchk1 in the depleted extracts is due to the absence of Claspin.

[0176] The kinase activity of recombinant Xchk1 that was isolated from the extracts that underwent the immunodepletion procedure was also examined. Consistent with the results described above, recombinant Xchk1 was activated about 4-fold in extracts containing poly(dA)$_{70}$-poly(dT)$_{70}$. This activation was strongly reduced in Claspin-depleted extracts, and was completely restored by the addition of recombinant His6-Claspin to these extracts. Taken together, these findings indicate that Claspin is required both for the checkpoint-dependent phosphorylation of Xchk1 and for the resulting increase in its kinase activity.

EXAMPLE 9

Claspin Is Necessary for the Delay of the Cell Cycle in Response to Incompletely Replicated DNA

[0177] In order to investigate the biological role of Claspin in the *Xenopus* system, cell cycle progression in Claspin-depleted egg extracts containing incompletely replicated DNA was examined. Claspin was removed from egg extracts with anti-Claspin antibodies and aphidicolin and demembranated *Xenopus* sperm chromatin was added to induce the formation of DNA replication blocks. Entry into mitosis was examined by monitoring the timing of nuclear envelope breakdown (NEB). Mmock-depleted extracts that had been treated with a control IgG or Claspin-depleted extracts to which recombinant His6-Claspin was added were treated in the same manner. Claspin-

depleted extracts containing aphidicolin inappropriately entered mitosis (half-maximal NEB at 150 min), indicating that the DNA replication checkpoint had been compromised. In contrast, mock-depleted extracts arrested well in interphase in the presence of aphidicolin. Likewise, aphidicolin-treated, Claspin-depleted extracts that were supplemented with His6-Claspin remained in interphase, indicating that the defect in the extracts lacking Claspin is due to the absence of this protein. Immunodepletion of Claspin had no effect on chromosomal DNA replication in egg extracts, as measured by incorporation of [α-^{32}P]dATP (Coleman *et al.* (1996) Cell *87*, 53-63). Thus, the compromised cell cycle delay in aphidicolin-treated extracts lacking Claspin cannot be attributed to a defect in the formation of DNA replication blocks (see Li and Deshaies, (1993) Cell *74*, 223-226).

[0178] Significantly, the Claspin-depleted extracts containing aphidicolin entered mitosis slower than control extracts lacking aphidicolin. A similar phenomenon was observed in Xchk1-depleted extracts suggesting that the aphidicolin-induced checkpoint in this system involves Xchk1-dependent and Xchk1-independent mechanisms (Kumagai *et al.*, (1998)). Overall, these findings indicate that removal of Claspin strongly compromises but does not completely eliminate the cell cycle delay that is triggered by DNA replication blocks. The response to aphidicolin is very similar in Xchk1-depleted extracts, which would be consistent with the interpretation that Claspin and Xchk1 act in the same pathway.

[0179] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.